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WO 99/14314 A WO 97/22703 A US 6013861 A
Planta Vol. 198 (3) 1996. Flipse E et.al. pages 340-347

(58) Field of Search
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(54) Abstract Title
Genetic modification of starch in plants

(57) There is described a transgenic plant which has reduced endogenous starch branching enzyme (SBE) activity and heterologous glucan branching (GBE) activity. Preferably endogenous SBE's are suppressed by antisense DNA. The invention also relates to plants having at least one species of starch in which the branching pattern is not wild-type. The expression of algal heterologous genes in potato is exemplified, particularly those from Gracilaria lemaneiformis.

GB 2 360 521

This print takes account of replacement documents submitted after the date of f with the formal requirements of the Patents Rules 1995

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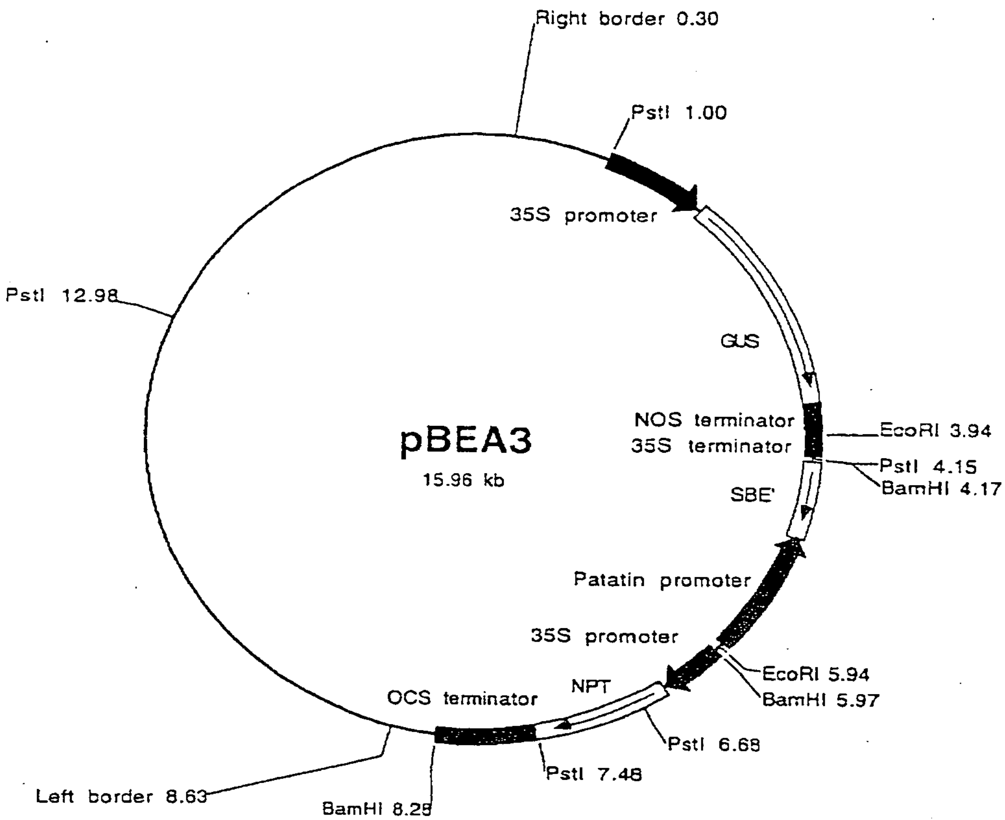
Figure 1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PstI 4.15
BamHI 4.17
SBE'
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35S promoter
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BamHI 5.97
NPT
OCS terminator
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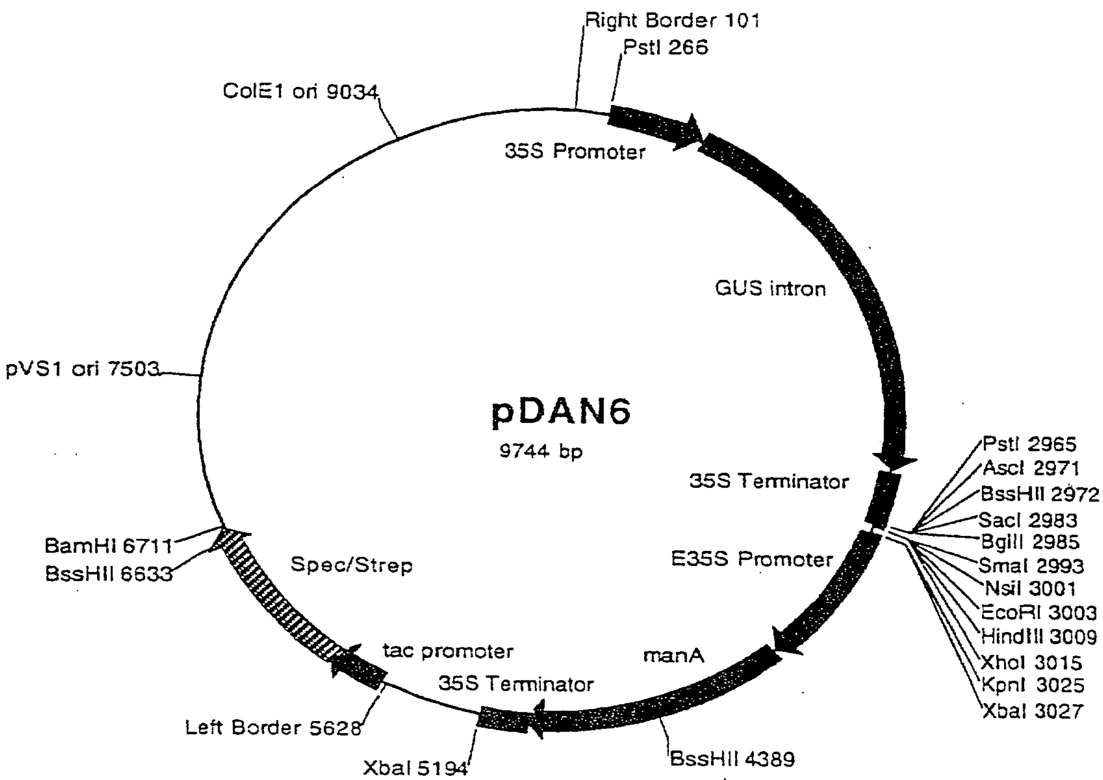
BamHI 8.28

Figure 2



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BssHII 2972
SacI 2983
BglII 2985
SmaI 2993
NsiI 3001
EcoRI 3003
HindIII 3009
XhoI 3015
KpnI 3025
XbaI 3027
E35S Promoter
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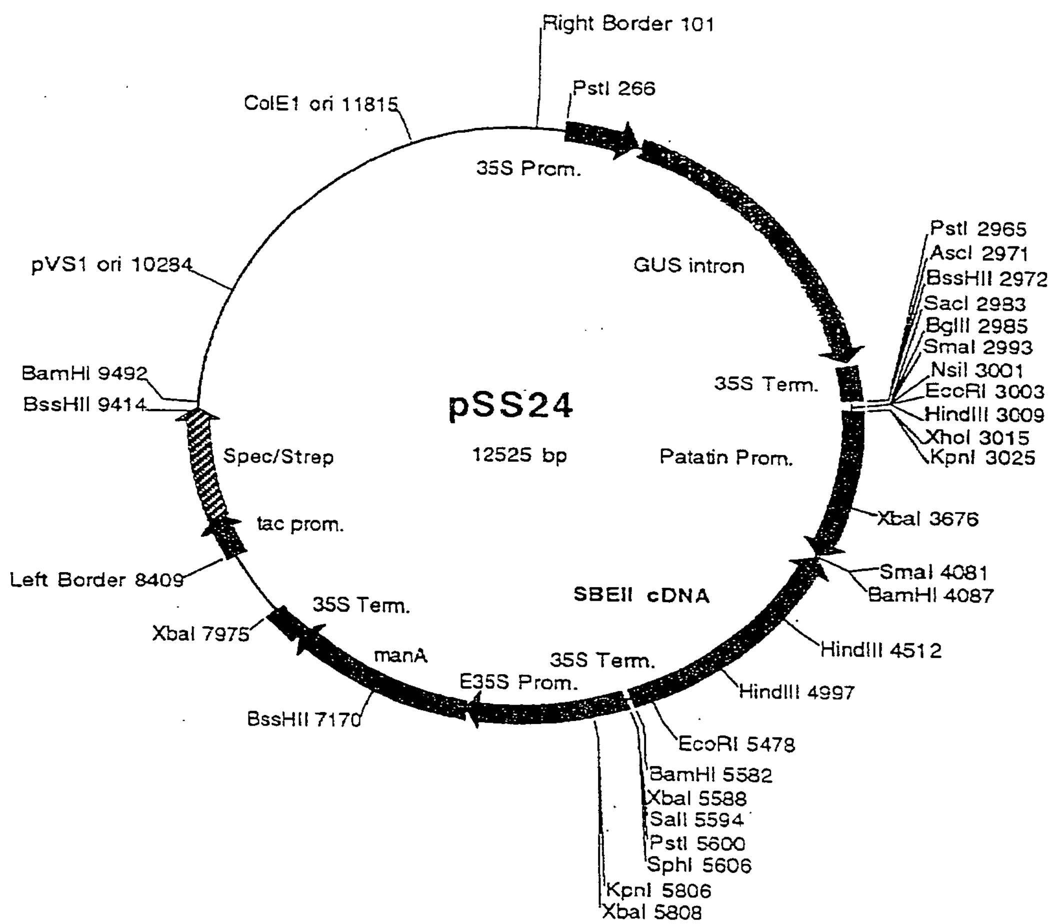
Figure 3



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NsiI 3001
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HindIII 3009
XhoI 3015
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SmaI 4081
BamHI 4087
HindIII 4512
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SalI 5594
PstI 5600
SphI 5606
KpnI 5806
XbaI 5808
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E35S Prom.
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BssHII 7170
XbaI 7975
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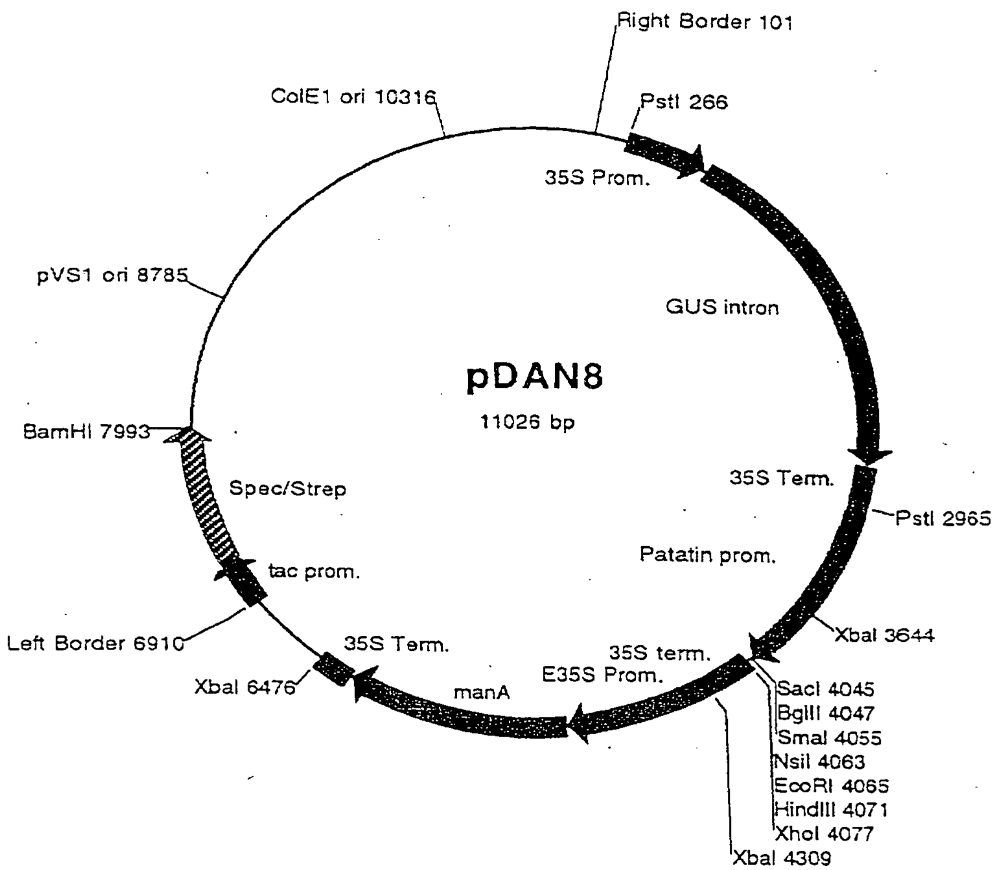
Figure 4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tac prom.
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35S term.
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XbaI 6476
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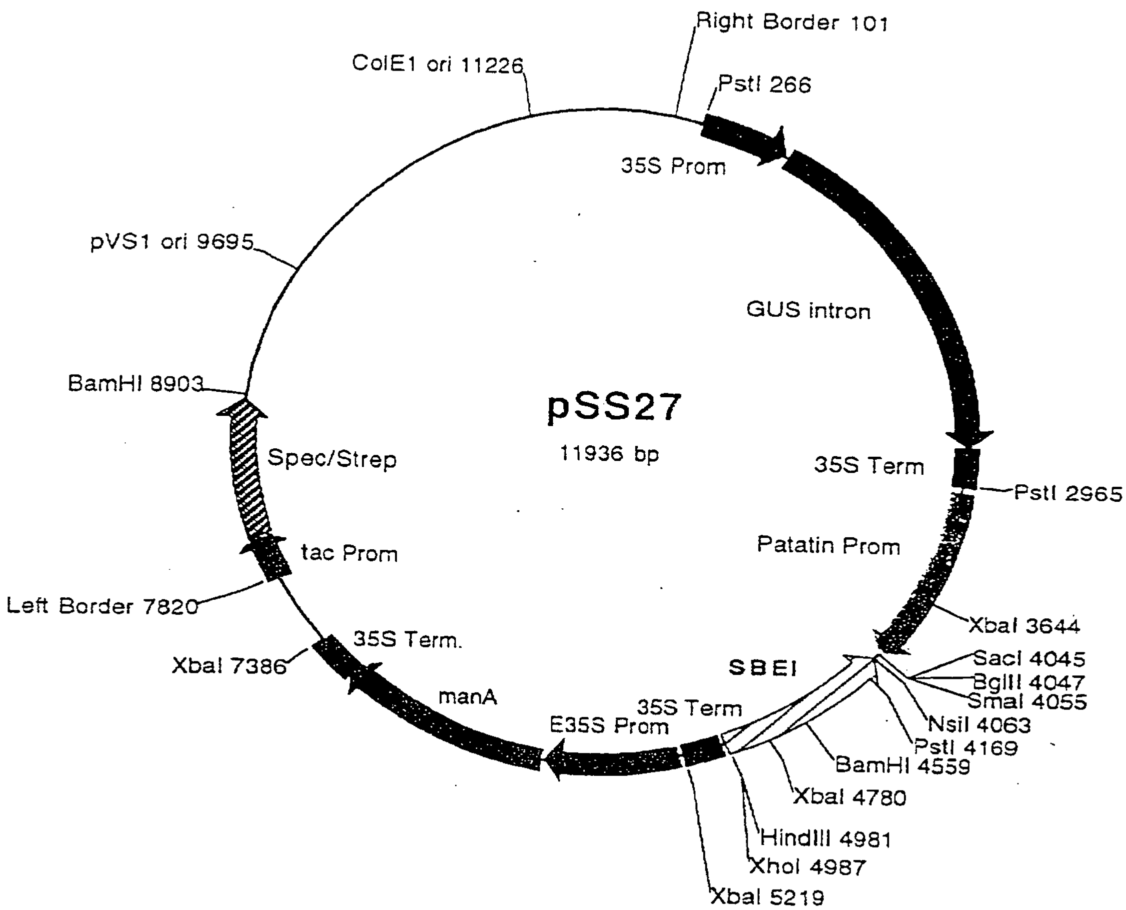
Figure 5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Patatin Prom
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SBEI
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BglII 4047
SmaI 4055
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manA
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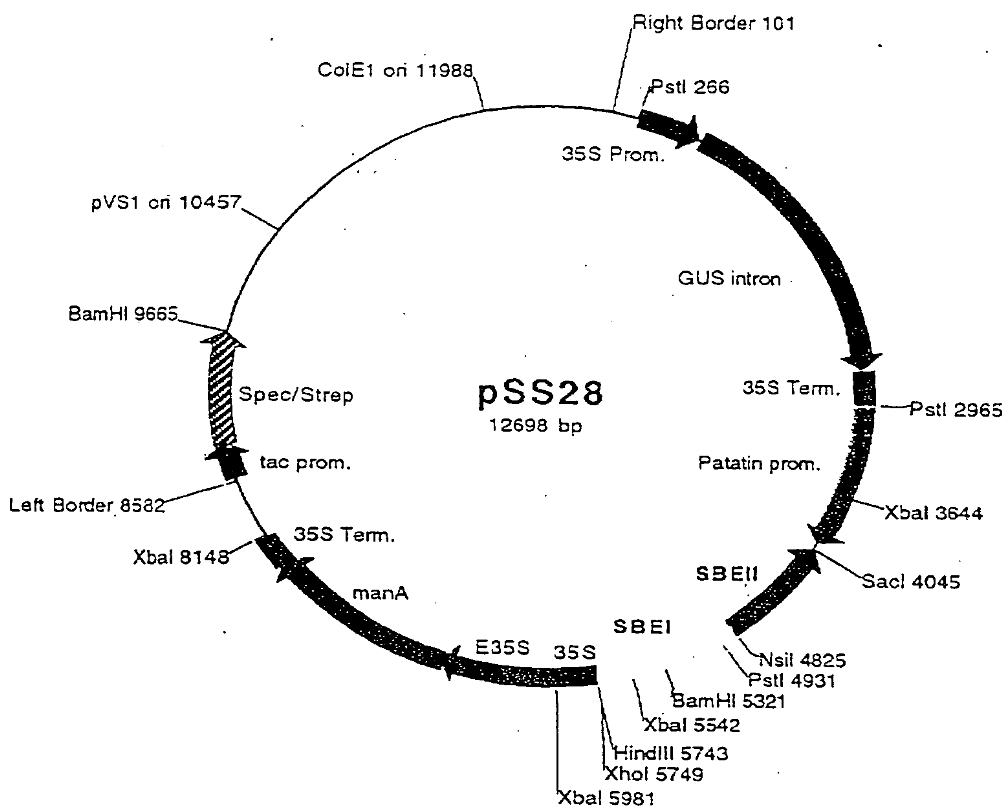
XhoI 4987
XbaI 5219

Figure 6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tac prom.
Patatin prom.
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XbaI 3644
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XbaI 8148
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SacI 4045
manA
SBEI
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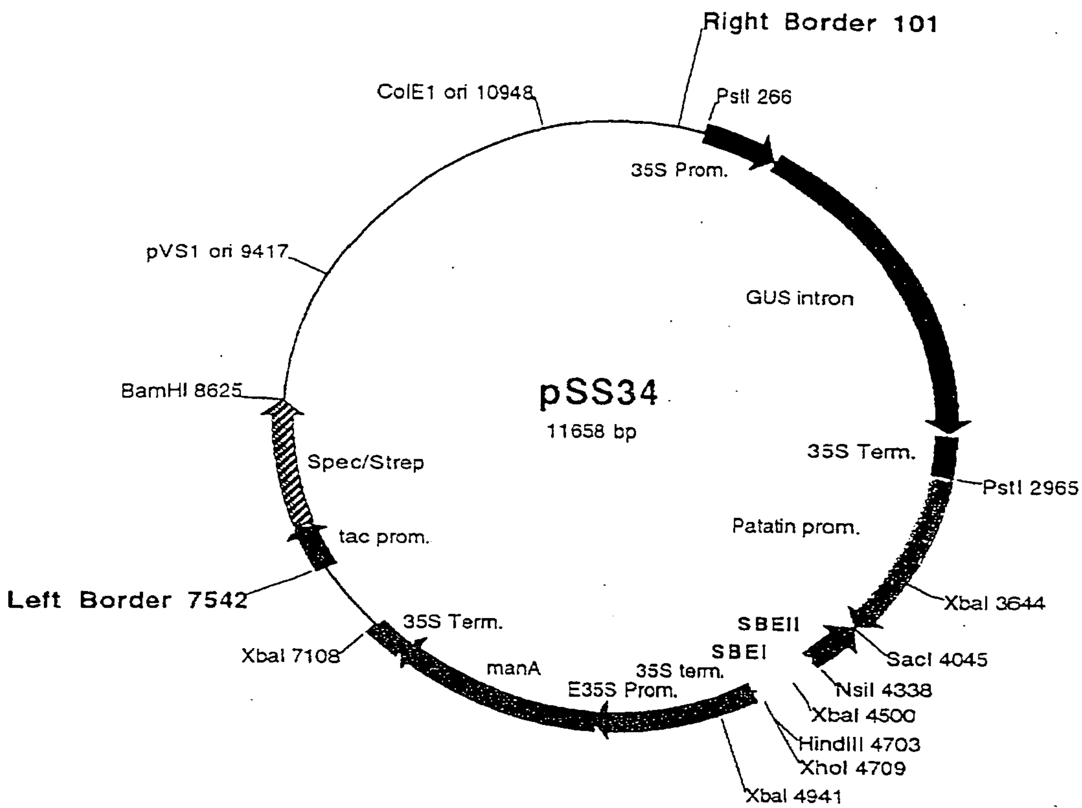
Figure 7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Spec/Strep
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tac prom.
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SBEI
SacI 4045
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NsiI 4338
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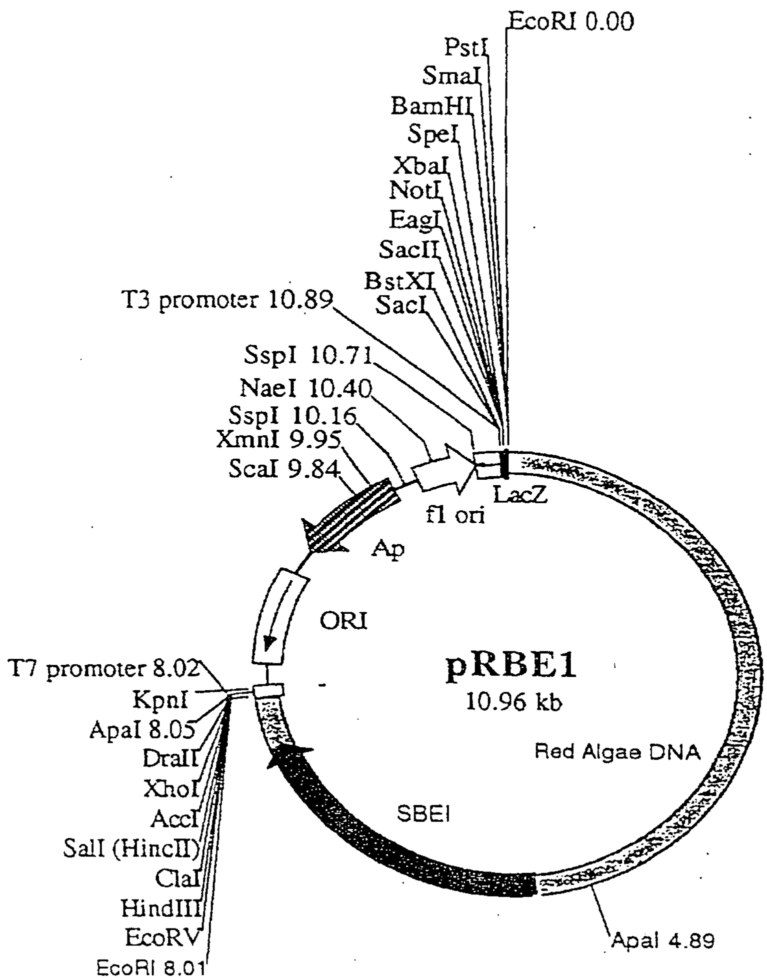
XbaI 4500
HindIII 4703
XhoI 4709
XbaI 4941

Figure 8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SmaI
BamHI
SpeI
XbaI
NotI
EagI
SacII
BstXI
SacI
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NaeI 10.40
SspI 10.16
XmnI 9.95
ScaI 9.84
LacZ
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Ap
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pRBE1
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Red Algae DNA
SBEI
T7 promoter 8.02
KpnI
ApaI 8.05
DraII
XhoI
AccI
SalI (HincII)
ClaI
HindIII
EcoRV
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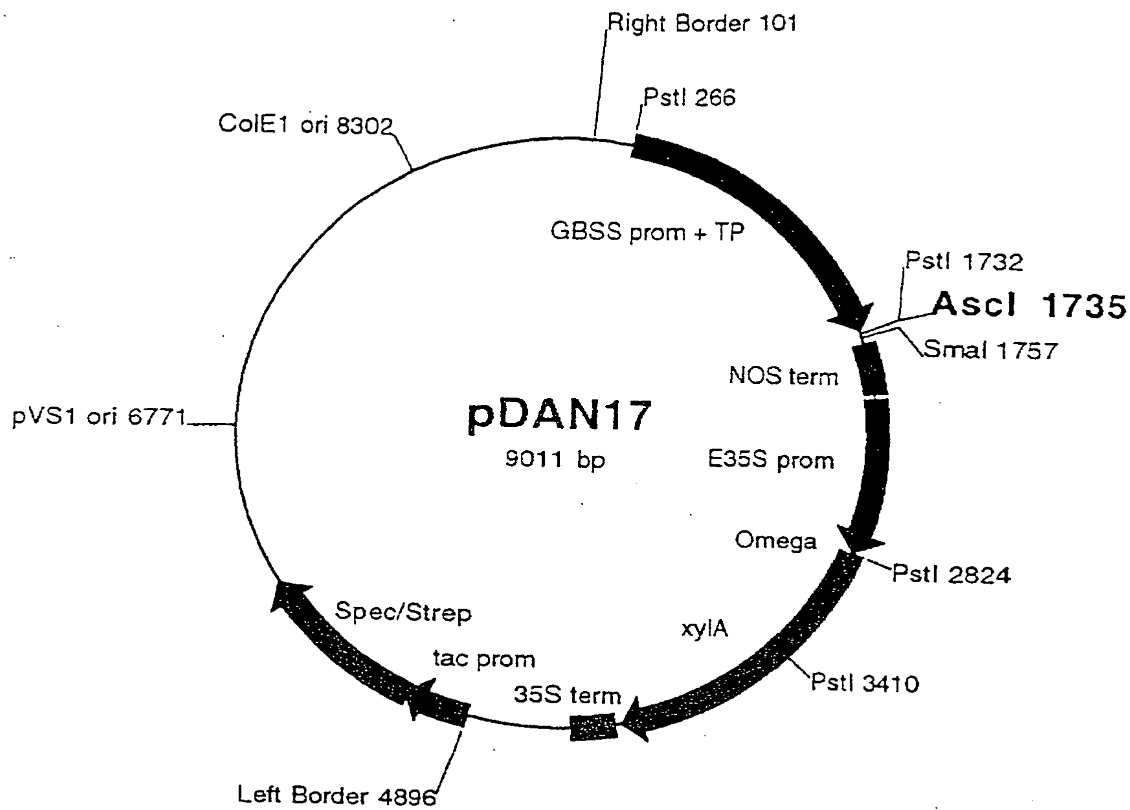
ApaI 4.89

Figure 9



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GBSS prom + TP
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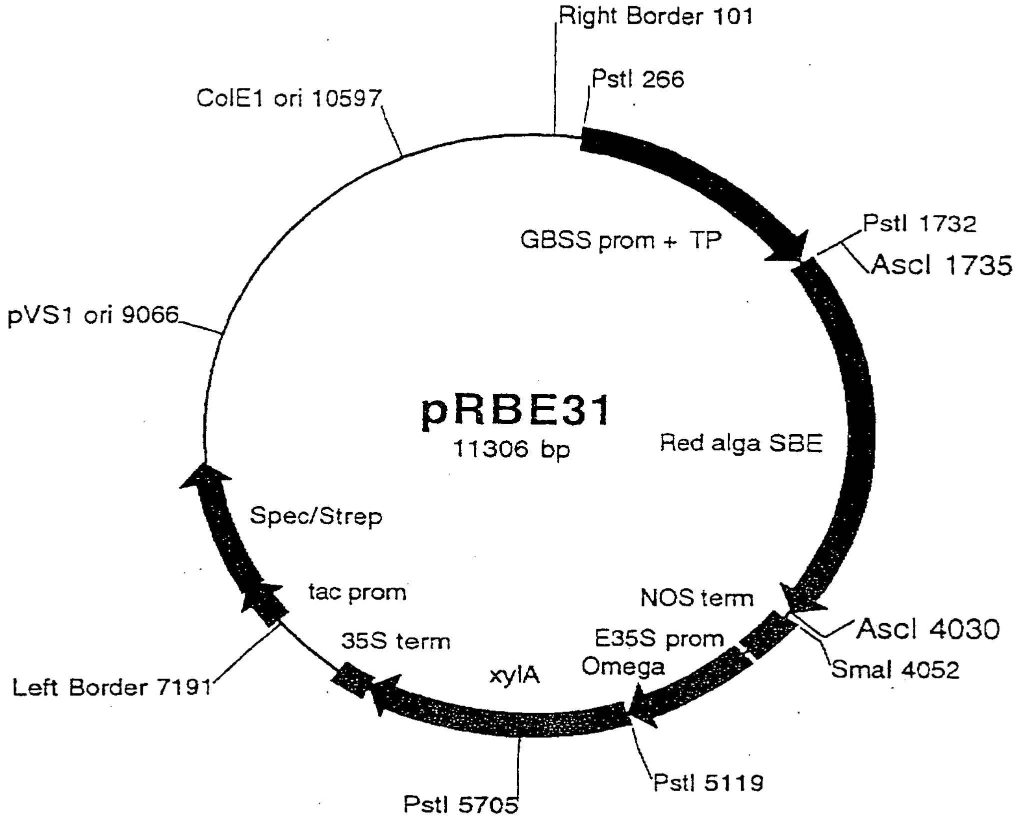
E35S prom
Omega
PstI 2824
Spec/Strep
xylA
tac prom
PstI 3410
35S term
Left Border 4896

Figure 10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Red alga SBE
Spec/Strep
NOS term
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Omega
SmaI 4052
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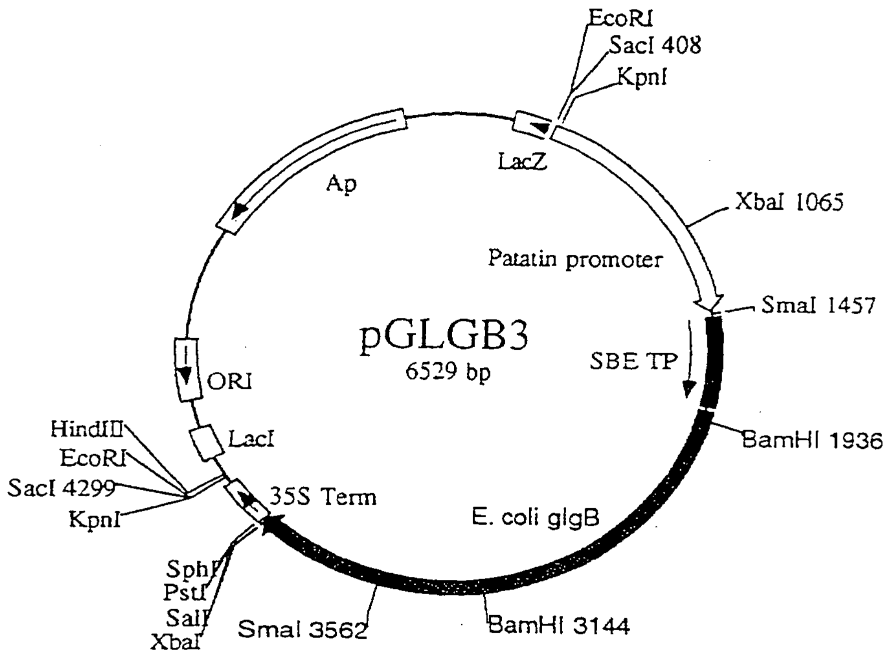
Figure 11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KpnI
LacZ
Ap
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ORI
HindIII
EcoRI
SacI 4299
KpnI
LacI
BamHI 1936
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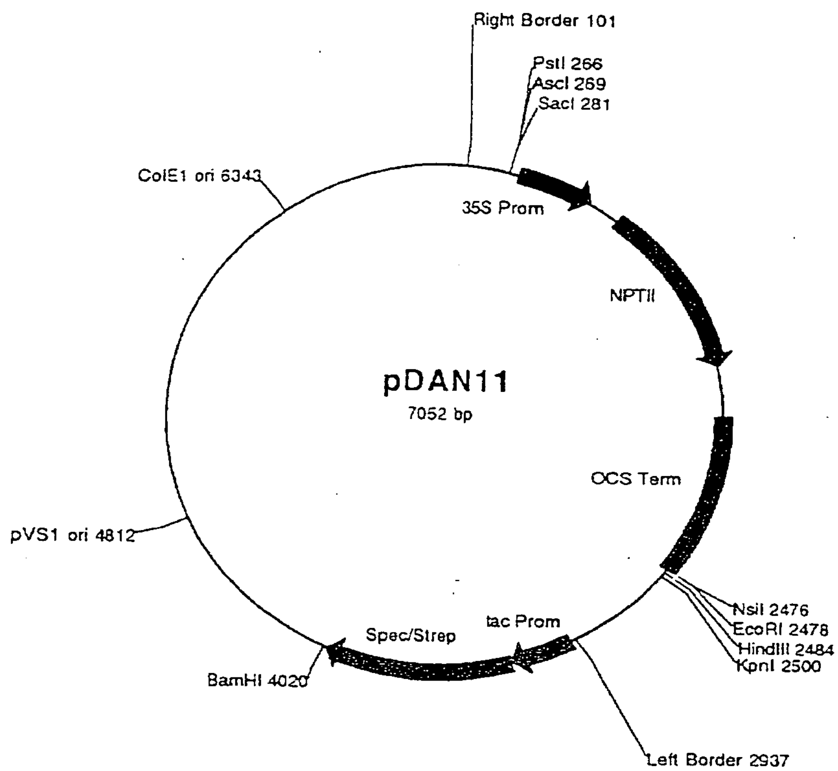
PstI
SalI
XbaI
SmaI 3562
BamHI 3144

Figure 12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BamHI 4020
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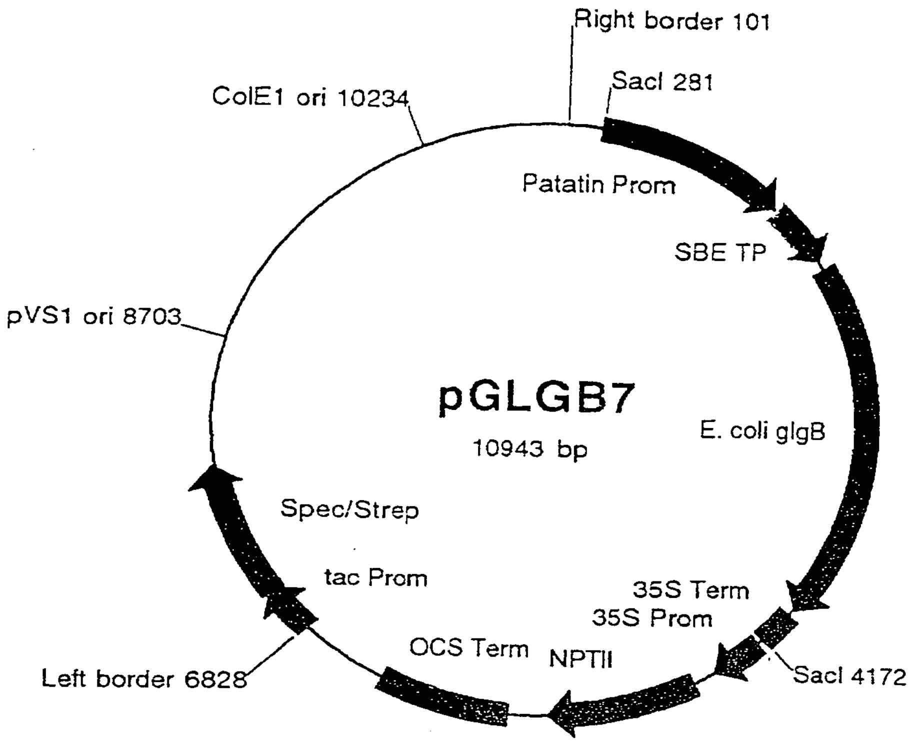
Figure 13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SacI 4172
Left border 6828

2360521

Enzyme

FIELD OF THE INVENTION

The present invention relates to plants with modified glucan branching enzyme (GBE) activity. The invention also related to starches produced from plants with modified GBE activity.

BACKGROUND TO THE INVENTION

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylopectin comprises linear or branched glucans. Amylose consists essentially of straight chains of α-1-4-linked glycosyl residues. Amylopectin (linear or branched glucans) comprises chains of α-1-4-linked glycosyl residues with some α-1-6 branches. The branched nature of amylopectin is created by the action of, among other things, enzyme(s) known as glucan branching enzyme(s) ("GBE(s)"). GBEs can catalyse the formation of branch points in the amylopectin (linear or branched glucans) molecule, for example by adding α-1,4 glucans through α-1,6-glucosidic branching linkages. GBEs include starch branching enzyme(s) (SBEs), as well as glycogen branching enzyme(s) (GLYBEs). Enzymes such as these may be from any source, for example from prokaryotic and/or eukaryotic sources.

It is known that starch is an important raw material. Starch is widely used in the food, paper, and chemical industries. Moreover, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties. However, such treatments are comparitively costly, and often involve hazardous chemicals. This is a problem associated with the prior art.

Within the past few years it has become desirable to make genetically modified plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In this regard, June Bourque provides a detailed summary of antisense strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149). Reviews on how enzymatic activity can be affected by expression of

particular nucleotide sequences may be found in the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3. Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity and/or introduce new enzymatic activity into the system.

SUMMARY OF THE INVENTION

As explained above, it is desirable to produce starches which have certain properties. Starches with these properties are not readily available in plants (in particular commercial crop plants) without post-harvest modification. It is surprisingly shown herein that plants may be genetically modified in order to produce starch with modified properties as compared with starch from the naturally occurring parent plants. The present application discloses that this may be accomplished by the alteration or modification of glucan branching enzyme (GBE) activity in plant tissues.

Accordingly, the present invention provides transformed plants which have modified glucan branching enzyme (GBE) activity. The term 'transfomed plants' includes transgenic plants, or plants harbouring transgene construct(s), whether stably or transiently.

GBE activity may be modified by any suitable technique, such as reducing the levels of GBE enzyme(s), such as through the use of an inhibitor of endogenous GBE expression and/or the use of an inhibitor of endogenous GBE replication (eg. transcription/translation); reducing the level of endogenous GBE enzymatic activity, such as through the use of a moiety that alters the active site of the endogenous GBE. Examples of such inhibitors or moieties include enzymes, antibodies, nucleotide sequences, etc. The term 'modified GBE activity' thus includes alteration by reduction of endogenous GBE and/or the introduction of heterologous GBE activity and/or the augmentation of endogenous GBE activity such as by modulation, enhancement, reduction or increase of said endogenous GBE activity. Preferably the reduction of endogenous GBE activity refers to the reduction of endogenous SBE activity. Preferably the term 'endogenous glucan branching enzyme' (endogenous GBE) refers to 'endogenous starch branching enzyme' (endogenous SBE).

In one preferred aspect, the alteration of glucan branching enzyme activity may be accomplished by reduction of endogenous GBE activity using antisense expression.

Preferably, this is accomplished using GBE antisense expression constructs as described hereinbelow, and as shown in the figures. Preferably 'GBE antisense expression constructs' refers to 'SBE antisense expression constructs'.

Antisense expression constructs are nucleic acid constructs which are capable of directing the production of RNA complementary to the coding RNA of a particular gene. Reduction of GBE activity refers to a reduction of the endogenous enzymatic activity, or to a reduction in the levels of endogenous GBE enzyme(s), or to a reduction in the levels of endogenous GBE transcript(s). This reduction of endogenous GBE activity may be accomplished by the use of sense intron expression, antisense intron expression, sense exon expression or antisense exon expression, or any combination thereof. In the case of a combination, the individual expression constructs may be on the same or different nucleic acid molecules, or any combination thereof. Preferably the reduction of endogenous GBE activity may be accomplished by the use of antisense exon expression. Preferably the reduction of endogenous GBE activity refers to the reduction of endogenous SBE activity by use of antisense exon expression, more preferably by use of antisense SBE exon expression.

The present invention also provides that plants having modified GBE activity may be produced by the expression of heterologous GBEs in the tissues of the plant(s). Preferably, plants having modified GBE activity may be produced by both the reduction of endogenous GBE activity using antisense expression, and the expression of heterologous GBEs in the tissues of said plant(s).

Accordingly, the present invention provides transformed plants which have modified glucan branching enzyme (GBE) activity, wherein said activity is brought about by the reduction of endogenous GBE activity, and the heterologous expression of one or more GBE(s).

DETAILED ASPECTS OF THE PRESENT INVENTION

In one aspect, the present invention relates to a transformed plant having an altered endogenous glucan branching enzyme (GBE) activity, and having a heterologous starch branching enzyme activity.

In another aspect, the present invention relates to a transformed plant having an altered endogenous glucan branching enzyme (GBE) activity, and having a heterologous starch branching enzyme activity, wherein said reduced GBE activity is effected via antisense expression of at least part of an GBE exon.

In another aspect, the present invention relates to a transformed plant having an altered endogenous glucan branching enzyme (GBE) activity, and having a heterologous starch branching enzyme activity, wherein said reduced GBE activity is effected via antisense expression of at least part of one or more SBE I exon(s), or antisense expression of at least part of one or more SBE II exon(s), or antisense expression of of at least part of one or more exon(s) from both SBE I and SBE II.

In another aspect, the present invention relates to a transformed plant having an altered endogenous glucan branching enzyme (GBE) activity, and having a heterologous starch branching enzyme activity, wherein said reduced GBE activity is effected via antisense exon expression, and wherein the antisense exon expression system and the heterologous GBE expression system are present as separate nucleic acid constructs.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises a SBE, or a glycogen branching enzyme (GLYBE), or both an SBE and a GLYBE.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises an GBE obtainable from an alga.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises an GBE obtainable from a red alga.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises an GBE obtainable from one or more algae selected from *Gracilaria gracilis* and *Gracilaria lemaneiformis*.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises a glycogen branching enzyme (GLYBE).

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises a glycogen branching enzyme (GLYBE), and wherein said GLYBE is obtainable from a bacterium.

In another aspect, the present invention relates to a transformed plant as described herein, wherein said heterologous GBE activity comprises a glycogen branching enzyme (GLYBE), and wherein said GLYBE is obtainable from the bacterium *Escherichia coli*.

In another aspect, the present invention relates to a method for producing starch with modified characteristics, said method comprising; providing a plant having reduced endogenous GBE activity, and having heterologous GBE activity, propagating said plant, and preparing starch from said plant.

In another aspect, the present invention relates to starch obtainable from a transformed plant as described herein.

In another aspect, the present invention relates to starch obtainable from a transformed plant as described herein, wherein said starch comprises at least one starch species having a branching pattern not naturally found in a wild-type parent plant. Preferably said starch comprises starch from a particular tissue, wherein at least one starch species from said tissue has a branching pattern not naturally found in a comparable tissue of a wild-type parent plant.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

Typically, the term 'altered' may mean 'modified' as explained above, such as reduced.

PREFERABLE ASPECTS

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. Examples of suitable promoters include the patatin promoter or the E35S promoter or the GBSS promoter. According to one aspect, preferably the promoter is the GBSS promoter.

GBE activity may be reduced using one or more appropriate nucleotide sequences relating to an GBE, such as an antisense GBE sequence or a part thereof and/or a sense GBE sequence or a part thereof. Examples of such sequences include one or more exons (or part thereof) and/or one or more introns (or part thereof). An organism can be transformed with these sequences by delivering those sequences on the same or different constructs (e.g. vectors).

Preferably, however, GBE activity is reduced using antisense SBE I expression, or antisense SBE II expression, or both antisense SBE I and antisense SBE II expression.

More preferably, GBE activity is reduced using both antisense SBE I and antisense SBE II expression.

ADVANTAGES

A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity.

Thus, the present invention provides a method of preparing particular starches in plants which could replace post-harvest modified starches.

Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a less detrimental effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

STARCH

Starch may occur in various different types. Normal potato starch refers to starch from organisms such as potatoes which do not have a modified GBE activity. Modified starch refers to starch from organisms such as potatoes according to the present invention which have an modified GBE activity.

High amylose starch is an example of a modified starch. High amylose starch has an modified branching pattern.. High amylose starch is produced when GBE activity is reduced. High amylose starch has a medium branched character. Floridean type starch is an example of a modified starch. Floridean type starch is produced when GBE activity is reduced, and a heterologous GBE (SBE) activity is present. Glycogen type starch is an example of a modified starch. Glycogen type starch is produced when GBE activity is reduced, and a heterologous GBE activity is present such as a glycogen branching enzyme (a GLYBE).

Starch types may be monitored microscopically, or by a simple viscosity measurement, or by gel filtration, or by amylose determination for example using iodine. Monitoring of starch types may be facilitated by the use of commonly available analytical devices such as the Rapid Visco Analyser (viscosity measurement), or the HPAEC Dionix (high performance anion exchange chromatography), or any other suitable device. Starch types may also be monitored using Nuclear Magnetic Resonance (NMR), preferably solid state NMR. Starch

types may also be monitored using mass spectrometry (MS). Starch types may also be monitored using any suitable assay known to those skilled in the art.

GLUCAN BRANCHING ENZYME ("GBE")

GBEs include starch branching enzyme(s) (SBEs), as well as glycogen branching enzyme(s) (GBEs). The term GBE as used herein relates to any glucan branching enzyme and therefore includes starch branching enzyme as well as glycogen branching enzyme (GLYBE) and similar enzymes.

STARCH BRANCHING ENZYME ("SBE")

SBE catalyses the formation of branch points in the amylopectin (linear or branched glucans) molecule by adding α-1,4 glucans through α-1,6-glucosidic branching linkages. The term SBE includes SBE I, SBE II as well as similar or related enzymes.

GLYCOGEN BRANCHING ENZYME (GLYBE)

Glycogen branching enzyme (GLYBE) is involved in glycogen metabolism. The term GLYBE includes similar or related enzymes.

NUCLEOTIDE SEQUENCES

The term "nucleotide" in relation to the present invention includes DNA and RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant DNA techniques.

The term "intron" is used in its normal sense as meaning a segment of nucleotides, usually DNA, that does not encode part or all of an expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides, usually DNA, encoding part or all of an expressed protein or enzyme.

Thus, the term "intron" refers to gene regions that are transcribed into RNA molecules, but which are spliced out of the RNA before the RNA is translated into a protein. In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in an organism, such as a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as any one of the antisense sequences described herein. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

Likewise, the terms "variant" or "homologue" or "fragment" in relation to a promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective promoter sequence providing the resultant promoter sequence allows expression of a gene of interest (GOI), preferably wherein the resultant promoter sequence has at least the same effect as those described herein. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide sequence encoding a GBE, or an antisense fragment thereof according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

The term "antisense" means a nucleotide sequence that is complementary to, and can therefore hybridize with, any one or all of the sequences of the present invention, including partial sequences thereof. Preferably, the antisense nucleic acids according to the present invention are not complementary to intron sequence(s). Unless otherwise stated, the term 'antisense sequence' as used herein means a nucleotide sequence that is antisense to at least part of an GBE exon.

According to the present invention, the antisense nucleic acid is preferably complementary to an entire exon of the gene to be inhibited. However, in some circumstances, partial antisense sequences may be used (i.e. sequences that are not or do not comprise the full complementary sequence) provided that the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences that are shorter than any one of the full sequences shown in the sequences disclosed herein, but which comprise nucleotides that are at least antisense to sense sequences found in respective exon or exons. Such partial sequences may be linked, fused, joined, concatenated or otherwise associated. Such sequences may comprise non-contiguous sections of one or more exons, such that for example one such sequence might comprise the end of one exon, linked with the beginning of another, discrete exon to form a single antisense nucleic acid, contigous or otherwise.

With regard to particular aspects of the present invention (i.e. specifically affecting GBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of a SBE gene, including complete or partial sequences thereof, providing the nucleotide sequences can affect GBE activity, preferably wherein the nucleotide sequences reduce or eliminate said GBE activity. Preferably, the nucleotide sequences of aspects of the present invention do not comprise an antisense intron sequence.

VECTORS

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E.Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the antisense nucleotide sequence aspect of the present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present

invention which includes direct or indirect attachment. The terms do not cover the natural combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

PROMOTERS

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the word in the art, e.g. an RNA polymerase binding site according to the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence(s) according to the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to modulate or affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

ADDITIONAL SEQUENCE OF INTEREST

In addition to reducing GBE activity, the present invention also encompasses transformation of the organism with one or more additional sequences of interest ("GOI"). These GOI(s) may be delivered on the same or different constructs.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues.

The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and α-galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for α-amylase, a protease or a glucanase.

In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase. Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

TRANSFORMED ORGANISMS

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

The term "parent plant" refers to a plant isogenic with one which gave rise to a particular transformed plant. The parent plant and its transformed derivative(s) will generally differ only in respect of the transgene(s) used in the creation of the transformed plant(s) from the parent plant. A transformed plant may proceed to become a parent plant for a further modified transformed plant.

The term "wild-type" has its normal meaning in the field of genetics, and may include particular cultivars or crop plant genotypes as appropriate which are not modified as taught herein.

If, for example, the organism is a plant then the promoter according to the present invention can be one that affects expression of the nucleotide sequence in any cell, tissue or organ, such as one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cassava; cereals such as wheat, maize, rice and barley; fruit; trees; flowers; and other plant crops. Preferably, the term means "potato".

The term "transformed organism" in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products obtained therefrom, and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide sequence of the present invention is incorporated in the genome of the organism. Preferably the transformed organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Sambrook *et al.* in Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

PRODUCTION OF TRANSFORMED PLANTS

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

A variety of transformation vectors are available for plant transformation and the SBE nucleotide sequences of the present invention can be used in conjunction with any such vectors. The selection of vector will depend on the preferred transformation technique and the plant species which is to be transformed. For certain target species, different selectable markers may be preferred.

For *Agrobacterium*-mediated transformation, binary vectors or vectors carrying at least one T-DNA border sequence are suitable. A number of vectors are available including pBIN19 (Bevan, Nucl. Acids Res. 12: 8711-8721 (1984), the pBI series of vectors, and pCIB10 and derivatives thereof (Rothstein *et al.* Gene 53: 153-161 (1987); WO 95/33818).

Binary vector constructs prepared for *Agrobacterium* transformation are introduced into an appropriate strain of *Agrobacterium tumefaciens* (for example, LBA 4044 or GV 3101) either by triparental mating (Bevan; Nucl. Acids Res. 12: 8711-8721 (1984)) or direct transformation (Höfgen & Willmitzer, Nucl. Acids Res. 16: 9877 (1988)).

For transformation which is not *Agrobacterium*-mediated (*i.e.* direct gene transfer), any vector is suitable and linear DNA containing only the construct of interest may be preferred. Direct gene transfer can be undertaken using a single DNA species or multiple DNA species (co-transformation; Schroder *et al.* Biotechnology 4: 1093-1096 (1986)).

Construction of vectors according to the invention employs conventional ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the constructs required. If desired, analysis to confirm correct sequences in the constructed plasmids is performed in a known fashion. Suitable methods for constructing expression vectors, preparing *in vitro* transcripts, introducing DNA into host cells, and performing analyses for assessing expression and function are known to those skilled in the art. Gene presence, amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA, dot blotting (DNA or RNA analysis), or *in situ* hybridisation, using an appropriately IGBElled probe which may be based on a sequence provided herein. Those skilled in the art will readily envisage how these methods may be modified, if desired.

DNA may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected cells may be prepared by transfecting cells with an expression vector having a selectable marker gene, and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, cells are transfected with a reporter gene to monitor transfection efficiency.

Heterologous DNA may be introduced into plant host cells by any method known in the art, such as electroporation or *Agrobacterium tumefaciens* mediated transfer. Although specific protocols may vary from species to species, transformation techniques are well known in the art for most commercial plant species.

In the case of dicotyledonous species, *Agrobacterium*-mediated transformation is generally a preferred technique as it has broad application to many dicotyledons species and is generally very efficient. *Agrobacterium*-mediated transformation generally involves the co-cultivation of *Agrobacterium* with explants from the plant and follows procedures and protocols that are known in the art. Transformed tissue is generally regenerated on medium carrying the appropriate selectable marker. Protocols are known in the art for many dicotyledonous crops including (for example) cotton, tomato, canola and oilseed rape, poplar, potato, sunflower, tobacco and soybean (see for example EP 0 317 511, EP 0 249 432, WO 87/07299, US 5,795,855).

In addition to *Agrobacterium*-mediated transformation, various other techniques can be applied to dicotyledons. These include PEG and electroporation-mediated transformation of protoplasts, and microinjection (see for example Potrykus *et al.* Mol. Gen. Genet. 199: 169-177 (1985); Reich *et al.* Biotechnology 4: 1001-1004 (1986); Klein *et al.* Nature 327: 70-73 (1987)). As with *Agrobacterium*-mediated transformation, transformed tissue is generally regenerated on medium carrying the appropriate selectable marker using standard techniques known in the art.

Until very recently there were very few reports on successful production of transformed monocotyledon crop plants. Although *Agrobacterium*-mediated transformation has been applied successfully to monocotyledonous species such as rice and maize and protocols for these approaches are available in the art, the most widely used transformation techniques for monocotyledons remain particle bombardment, and PEG and electroporation-mediated transformation of protoplasts. This relatively slow development within monocots was due to two causes. Firstly, until the early 1980s, efficient regeneration of plants from cultured cells and tissues of monocots had proven very difficult. This problem was ultimately solved by the culture of explants from immature and embryogenic tissue, which retain their morphogenic potential on nutrient media containing plant growth regulators. Secondly, the monocots are not a natural host for *Agrobacterium tumefaciens*, meaning that the successful developed techniques within the dicots using their natural vector *Agrobacterium tumefaciens* was unsuccessful for many years in the monocots.

Nevertheless, it is now possible to successfully transform and produce fertile transformed plants of maize using methods such as: (1) Silicon Carbide Whiskers; (2) Particle Bombardment; (3) DNA Uptake by PEG treated protoplast; or (4) DNA Uptake in Electroporation of Tissue. Each of these methods - which are reviewed by Thompson (1995 Euphtytica 85 pp 75-80) - may be used to prepare *inter alia* transformed plants according to the present invention.

In particular, the particle Gun method has been successfully used for the transformation of monocots. However, EP-A-0604662 reports on a different method of transforming monocotyledons. The method comprises transforming cultured tissues of a monocotyledon under or after dedifferentiation with *Agrobacterium* containing a super binary vector as a selection means a hygromycin-resistant gene was used. Production of transformed calli and plant was demonstrated using the hygromycin selection. This

method may be used to prepare *inter alia* transformed plants according to the present invention.

In the case of maize, Gordon-Kamm *et al.* (Plant Cell 2: 603-618 (1990)), Fromm *et al.* (Biotechnology 8: 833-839 (1990) and Koziel *et al.* (Biotechnology 11: 194-200 (1993)) have published techniques for transformation using particle bombardment.

In the case of rice, protoplast-mediated transformation for both *Japonica*- and *Indica*-types has been described (Zhang *et al.* Plant Cell Rep. 7: 379-384 (1988); Shimamoto *et al.* Nature 338: 274-277; Datta *et al.* Biotechnology 8: 736-740 (1990)) and both types are also routinely transformable using particle bombardment (Christou *et al.* Biotechnology 9: 957-962 (1991)).

In the case of wheat, transformation by particle bombardment has been described for both type C long-term regenerable callus (Vasil *et al.* Biotechnology 10: 667-674 (1992)) and immature embryos and immature embryo-derived callus (Vasil *et al.* Biotechnology 11: 1553-1558 (1993); Weeks *et al.* Plant Physiol. 102: 1077-1084 (1993)). A further technique is described in published patent applications WO 94/13822 and WO 95/33818.

SELECTABLE MARKERS

Transformation of plant cells is normally undertaken with a selectable marker which may provide resistance to an antibiotic or to a herbicide. Selectable markers that are routinely used in transformation include the *nptII* gene which confers resistance to kanamycin (Messing & Vierra Gene 19: 259-268 (1982); Bevan *et al.* Nature 304: 184-187 (1983)), the *bar* gene which confers resistance to the herbicide phosphinothricin (White *et al.* Nucl. Acids Res. 18: 1062 (1990); Spencer *et al.* Theor. Appl. Genet. 79: 625-631 (1990)), the *hph* gene which confers resistance to the antibiotic hygromycin (Blochlinger & Diggelmann Mol. Cell Biol. 4: 2929-2931 (1984)), and the *dhfr* gene which confers resistance to methotrexate (Bourouis *et al.* EMBO J 2: 1099-1104 (1983)). More recently, a number of selection systems have been developed which do not rely of selection for resistance to antibiotic or herbicide. These include the inducible isopentyl transferase system described by Kunkel *et al.* (Nature Biotechnology 17: 916-919 (1999), or the mannose system or xylose system (described in more detail below).

CODON PREFERENCE

The nucleic acid constructs of the invention are suitable for expression in a variety of different organisms. However, to enhance the efficiency of expression it may be necessary to modify the nucleotide sequence encoding the GBE(s) to account for different frequencies of codon usage in different host organisms. Hence it is preferable that the sequences to be introduced into organisms, such as plants, conform to preferred usage of codons in the host organism.

In general, high expression in plants is best achieved from codon sequences that have a GC content of at least 35% and preferably more than 45%. This is thought to be because the existence of ATTTA motifs destabilize messenger RNAs and the existence of AATAAA motifs may cause inappropriate polyadenylation, resulting in truncation of transcription. Murray *et al.* (Nucl. Acids Res. 17: 477-498 (1989)) have shown that even within plants, monocotyledonous and dicotyledonous species have differing preferences for codon usage, with monocotyledonous species generally preferring GC richer sequences. Thus, in order to achieve optimal high level expression in plants, gene sequences can be altered to accommodate such preferences in codon usage in such a manner that the codons encoded by the DNA are not changed.

Plants also have a preference for certain nucleotides adjacent to the ATG encoding the initiating methionine and for most efficient translation, these nucleotides may be modified. To facilitate translation in plant cells, it is preferable to insert, immediately upstream of the ATG representing the initiating methionine of the gene to be expressed, a "plant translational initiation context sequence". A variety of sequences can be inserted at this position. These include the sequence the sequence 5'-AAGGAGATATAACAATG-3' (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)), the sequence 5'-GTCGACCATG-3' (Clontech 1993/1994 catalog, page 210), and the sequence 5'-TAAACAATG-3' (Joshi *et al.* Nucl. Acids Res. 15: 6643-6653 (1987)). For any particular plant species, a survey of natural sequences available in any databank (*e.g.* GenBank) can be undertaken to determine preferred "plant translational initiation context sequences" on a species-by-species basis.

Any changes that are made to the coding sequence can be made using techniques that are well known in the art and include site directed mutagenesis, PCR, and synthetic gene construction. Well known protocols for transient expression in plants

can be used to check the expression of modified genes before their transfer to plants by transformation.

Thus, in summary, the present invention relates to a transformed organism, having a reduced endogenous glucan branching enzyme (GBE) activity, and having a heterologous glucan branching enzyme activity. Preferably the transformed organism is a transformed plant.

In a preferred aspect, the present invention relates to a transformed organism having a reduced endogenous glucan branching enzyme (GBE) activity, and having a heterologous glucan branching enzyme activity, wherein said reduced GBE activity is effected via expression of a nucleotide sequence that is antisense to at least part of an GBE exon.

In a more preferred aspect, the present invention relates to a transformed organism having a reduced endogenous glucan branching enzyme (GBE) activity, and having a heterologous glucan branching enzyme activity, wherein said reduced GBE activity is effected via expression of a nucleotide sequence that is antisense to at least part of an GBE exon, and wherein said organism is a plant.

The present invention will now be described, by way of example only, in which reference will be made to the following figures:

Figure 1, which shows a plasmid.
Figure 2, which shows a plasmid.
Figure 3, which shows a plasmid.
Figure 4, which shows a plasmid.
Figure 5, which shows a plasmid.
Figure 6, which shows a plasmid.
Figure 7, which shows a plasmid.
Figure 8, which shows a plasmid.
Figure 9, which shows a plasmid.
Figure 10, which shows a plasmid.
Figure 11, which shows a plasmid.
Figure 12, which shows a plasmid.
Figure 13, which shows a plasmid.

Examples

Example1 – Reduction of endogenous starch branching enzyme activity

Glucan branching enzyme (GBE) activity is reduced in plants by expressing antisense GBE nucleic acids in said plants. This is accomplished using SBE I antisense expression, or using SBE II antisense expression, or using SBE I and SBE II antisense expression, as discussed herein.

Reduction of starch branching enzyme activity by antisense SBE I expression.

Nucleic acid constructs for the expression of antisense SBE are prepared.

A 692 bp EcoRI fragment from the 5' end of a potato starch branching enzyme I (SBEI) cDNA (Poulsen and Kreiberg (1993) Plant physiol 102:1053-1054) is inserted in antisense orientation after a patatin class I promoter in plasmid pPATA1 (see WO94/24292). From the resulting plasmid, pGBE3, the antisense SBEI cassette is isolated as a 1996 bp EcoRI restriction fragment, which is inserted into the plant transformation vector pBKL4 (see WO94/24292) yielding plasmid pBEA3 (shown in Fig. 1).

Potato plants are transformed using kanamycin resistance as the selectable marker, according to techniques well known in the art, and as discussed herein, and transformed plants are isolated.

These transformed plants have reduced GBE activity.

Reduction of starch branching enzyme activity by antisense SBE II expression.

Nucleic acid constructs for the expression of antisense SBE are prepared.

A potato SBE II cDNA clone is obtained by RT-PCR from potato sprout total RNA using the primers 5' – TCA GCA GTA ATG GTG ATC GGA GG – 3' and 5' – CAC AAG TTC GTT CAT TCT TCT TCT AC -3' and a Titan One Tube RT-PCR Kit (Boehringer Mannheim). The RT-PCR program is as follows: 50 °C for 30 minutes, 95 °C for 4 minutes, 35 cycles of 92 °C for 30 seconds, 62 °C for 45 seconds, and 68 °C for 2 minutes, and a final elongation step of 68 °C for 7 minutes. The

resulting 2.6 kb cDNA fragment is inserted into pCR2.1-TOPO to give plasmid pSS19.1. The nucleotide sequence of the SBE II cDNA is shown in SEQ. ID. NO. 1.

Using plasmid pSS19.1 as DNA template a 1495 bp SBE II DNA fragment from the 5' end of the SBE II cDNA is PCR amplified with the primers 5' – CGG GAT CCC GTC AGC AGT AAT GGT GAT CGG AGG – 3' and 5' – CGG GAT CCC GAC CGA TAA TCC GTG GTG AG. This fragment is inserted as a BamHI fragment in an antisense orientation after the patatin promoter in plasmid pPATA1 (see WO94/24292) to give plasmid pSS21.

The SBE II antisense cassette from this plasmid pSS21 is isolated as a 2781 bp KpnI fragment and inserted in the KpnI site of pDAN6 (Fig. 2) yielding pSS24 (Fig. 3).

Potato plants are transformed using the mannose selection principle (see United States Patent No. 5,767,378).

A number of transformed plants are isolated.

These transformed plants have reduced GBE activity.

Reduction of starch branching enzyme activity by antisense SBE I and antisense SBE II expression.

Nucleic acid constructs for the expression of antisense SBE I and SBE II are prepared.

A 918 bp fragment is amplified from the SBE I cDNA (see above) by PCR using the primers 5' – CCC AAG CTT CCC GTC TGT AAG CAT CAT TAG TG – 3' and 5' – CCA ATG CAT AGG GCG AGG GTA TTT GAA GTG G – 3'. The PCR fragment is digested with NsiI and HindIII and inserted into these sites in pDAN8 (Fig. 4) to give plasmid pSS27 (Fig. 5).

A 5' end potato SBE II cDNA is obtained by RT-PCR from potato sprout total RNA using the primers 5' – TTG ATG GGG CCT TGA ACT CAG C – 3' and 5' – ACC CTC ATA CTT GTC AAT TGC CTC – 3' and a Titan One Tube RT-PCR Kit (Boehringer Mannheim). The RT-PCR program is as follows: 50 °C for 30 minutes, 95 °C for 5 minutes, 35 cycles of 92 °C for 45 seconds, 66 °C for 45 seconds, and

68 °C for 1 minutes, and a final elongation step of 68 °C for 7 minutes. Two slightly different 0.7 kb cDNA fragments are obtained and inserted in pCR2.1-TOPO to give plasmids pSS25 and pSS26. The nucleotide sequences of these 5' end SBEII cDNAs are shown in SEQ. ID. NO. 2 and SEQ. ID. NO. 3.

Using plasmid pSS25 as DNA template a SBE II DNA fragment with SacI and NsiI restriction sites at the ends is generated by PCR using the primers 5' – TAG GCG AGC TCA CCC TCA TAC TTG TCA ATT GCC TC – 3' and 5' – CCA ATG CAT TTG ATG GGG CCT TGA ACT CA – 3'. The SacI and NsiI restriction fragment is then inserted into pSS27 (described above-see Fig. 5) yielding pSS28 (Fig. 6).

This plasmid is used for inhibition of SBE I and SBE II expression in potato tubers by transforming potato plants using the mannose selection principle (see United States Patent No. 5,767,378).

Another plasmid (pS34) useful for inhibition of SBE I and SBE II expression in potato tubers is also constructed. This plasmid is similar to plasmid pSS28 (described above), but includes only 345 bp of 5' end SBE I cDNA and 277 bp of 5' end SBE II cDNA. pSS34 is constructed by production of a SBE I cDNA fragment by PCR using the primers 5' - CCC AAG CTT CCC GTC TGT AAG CAT CAT TAG TG – 3' and 5' - CCA ATG CAT AGC GGA AAT AGC TGA ACT GTG CTT CAT C – 3'.
The resulting NsiI and HindIII restriction fragment is inserted in pDAN8 digested with the same restriction enzymes yielding pSS31. Similarly, the SBE II cDNA fragment is generated using the primers 5' - CCA ATG CAT TTG ATG GGG CCT TGA ACT CA – 3' and 5'- TAG GCG AGC TCG CCA AGA TGT GAA AGA GAG TGC – 3' and the resulting PCR fragment is inserted as a SacI-NsiI restriction fragment in pSS31 to give pSS34 (Fig. 7).

Potato plants are transformed using the mannose selection principle (see United States Patent No. 5,767,378).

A number of transformed plants are isolated.

These transformed plants have reduced GBE activity.

Example 2 – Cloning of genes encoding glucan branching enzymes (GBEs)

GBE genes are isolated for heterologous expression in organisms according to the present invention, such as plants.

In this Example, an GBE gene is isolated from the red alga *Gracilaria lemaneiformis*. In this Example, the GBE gene isolated is a SBE gene.

A pair of degenerated PCR primers (5' – (TC)T(GATC)ATGGC(GATC)AT(ATC)ATGGA(GA)CA – 3' and 5' – CC(GA)TC(GA)AA(GATC)CG(GA)AA(GATC)CC(GA)TC – 3') are designed from two conserved amino acid motifs in starch branching enzymes (SBEs). These motifs are the LMAIMEH and FDGFRFDG, found in the central region of the enzymes.

The PCR primers and chromosomal *Gracilaria lemaneiformis* DNA are used in a PCR amplification with Taq DNA polymerase. The PCR program is as follows: denaturation at 94 °C for 2 minutes, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 42 °C for 1 minutes, and extension at 72 °C for 2 minutes, followed by one extension step at 72 °C for 7 minutes.

The fragments obtained are inserted into pCR-SCRIPT SK (+) (Stratagene, USA) and sequenced. One of the fragments is found to have the desired branching enzyme sequences (SEQ. ID. NO. 4). The 352 bp insert is then used as a radioactive DNA hybridisation probe for screening of a genomic *Gracilaria lemaneiformis* ZAP II library (Bojsen et al. (1999) Biochimica et Biophysica acta 1430:396-402) using standard protocols (Sambrook et al. (1989) Molecular cloning: a laboratory manual, Second edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

One of the clones obtained is excised *in vivo* using the protocols provided by Stratagene (USA) yielding pRBE1 (Fig. 8), which is the pBluescript SK (-) vector containing an 8 kb red algae genomic DNA. The branching enzyme gene is located on a 3.5 kb ApaI DNA fragment which is sequenced (shown as SEQ. ID. NO. 5).

The nuclectide sequence reveals an open reading which encodes a 760 amino acid polypeptide with high similarity to plant starch branching enzymes and eukaryotic glycogen branching enzymes.

Thus, an alignment of the proposed red algae branching enzyme with the mature forms of potato starch branching enzyme I and potato starch branching enzyme II reveals 375 identites (44 %) and 367 identites (42 %), respectively. This strongly suggests that the cloned gene is a red algae branching enzyme. A comparison with the *E. coli* glycogen branching enzyme shows that the two enzymes have 142 identities (16 %). Thus, the cloned algae branching enzyme is closely related to the plant starch branching enzymes. Since the algae enzyme lacks the 11 amino acid motif found in the proposed 8th loop of the class A plant starch branching enzymes (Burton et al. (1995) The Plant Journal 7(1), 3-15) the enzyme is most likely a class B type of branching enzyme.

Recently, a gene for a starch branching enzyme was isolated from the red alga *Gracilaria gracilis* (Lluisma & Ragan (1998) Curr Genet 34:105-111). The *G. lemaneiformis* and *G. gracilis* genes show significant sequence similarity in the coding regions.

A comparison of the encoded proteins shows that the two branching enzymes have 616 identical amino acids (80 % similarity/identity). The differences between the two enzymes are mainly found in the N- and C- termini. Thus, the two branching enzymes may be two isoforms with differing enzymatic activities.

In this way, genes encoding GBEs may be isolated from a variety of sources for use in the present invention.

Example 3 –Heterologous expression of starch branching enzymes

The present invention provides for the heterologous expression in plants of GBEs from various different species.

Heterologous expression of algal GBEs in plants

In this Example, the heterologous expression of algal GBE in a higher plant is disclosed. In this example, the algal GBE is algal SBE.

A nucleic acid construct for expression of a starch branching enzyme from the red alga *Gracilaria lemaneiformis* in potato tubers is produced as follows:

The gene encoding the *Gracilaria lemaneiformis* branching enzyme is amplified by PCR using the primers 5' – GGC GCG CCG GGC TCG GAA GAC CC – 3' and GGC GCG CCT CAC ACA GCT TCC TTC TG – 3' with pRBE1 as DNA template.

The PCR fragment is inserted in pCR2.1-TOPO (Invitrogen, The Netherlands) yielding pRBE28. From this plasmid the starch branching enzyme gene is isolated as an AscI restriction fragment and inserted in the AscI site of the plant transformation vector pDAN17 (Fig. 9) after the GBSS promoter and GBSS transit peptide resulting in pRBE31 (Fig. 10).

This plasmid is used for transformation of potato plants using the xylose selection principle (Haldrup et al. (1998) Plant Mol Biol 37:287-296).

A number of transformed plants are isolated which heterologously express the *Gracilaria lemaneiformis* SBE.

These transformed plants have modified GBE activity.

Heterologous expression of bacterial GBEs in plants

In this Example, the heterologous expression of bacterial GBE in a higher plant is disclosed.

A nucleic acid construct for expression of an glucan branching enzyme (a glycogen branching enzyme (GLYBE)) from the bacterium *Escherichia coli* in potato tubers is produced as follows:

The *E. coli glgB* gene is amplified from λ-phage 616 from the Kohara *E. coli* λ-phage collection (Kohara et al. (1987) Cell 50:495-508) using the primers: 5' - GAA GAT CTA TCC GAT CGT ATC GAT AGA GAC – 3' and 5' - GAA GAT CTA TCA TTC TGC CTC CCG AAC C – 3'. The PCR program is: 95 °C for 5 minutes, 25 cycles of 95 °C for 1 minutes, 55 °C for 1 minutes, and 75 °C for 3 minutes, followed by a 10 minutes elongation step at 75 °C. *Pfu* DNA polymerase (Stratagene, USA) is used in the PCR.

The 2184 bp PCR product is digested with BglII and inserted in pBETP5 (see WO 94/24292), and cut with BamHI to give pGLGB3 (Fig. 11). The 3891 bp SacI fragment from pGLGB3 is then inserted in the SacI site of pDAN11 (Fig. 12) yielding pGLGB7 (Fig. 13).

This plasmid is used for transformation of potato plants using the appropriate selectable marker as described above.

A number of transformed plants are isolated which heterologously express the *Escherichia coli* GBE glycogen branching enzyme (GLYBE).

These transformed plants have modified GBE activity.

Example 4 - Production of floridean starch types in potatoes.

The invention provides for the production in certain plants of starch types which are not naturally found in such plants.

In this Example, the invention provides for the production of floridean starch types in potatoes.

This is accomplished in potato plants by reducing endogenous GBE activity *via* the use of antisense construct expression, and by providing modified GBE activity *via* heterologous expression of algal GBE (in this example, the algal GBE is algal SBE) as follows.

Repression of endogenous SBE I and SBE II expression by transformation of potato plants with plasmid pSS28 or plasmid pSS34 as described in Example 1.

Mannose selection is used in the transformation of potato plants, and transformed plants are isolated.

These transformed plants have reduced GBE activity.

A nucleic acid construct for expression of a starch branching enzyme from the red alga *Gracilaria lemaneiformis* is produced as in Example 3.

This construct (pRBE31) is used for transformation of the transformed potato plants with reduced GBE activity using the xylose selection principle (Haldrup et al. (1998) Plant Mol Biol 37:287-296).

A number of transformed plants are isolated which heterologously express the *Gracilaria lemaneiformis* SBE.

These transformed plants have modified GBE activity.

Starch is prepared from these transformed plants having modified GBE activity, and is found to comprise floridean starch types.

Example 5 - Production of "floridean starch types" in potatoes.

The invention provides for the production in certain plants of starch types which are not naturally found in such plants.

In this Example, the invention provides for the production of floridean starch types in potatoes.

This is accomplished in potato plants by reducing endogenous GBE activity *via* the use of antisense construct expression, and by providing modified GBE activity *via* heterologous expression of algal SBE as follows.

First, repression of endogenous SBE II expression is brought about by transformation of potato plants with plasmid pSS24 as described in Example 1.

Mannose selection is used in the transformation of potato plants, and transformed plants are isolated.

These transformed plants have reduced SBE II activity.

Next, repression of endogenous SBE I expression is brought about by transformation of the SBE II repressed plants with plasmid pBEA3 as described in Example 1.

Kanamycin selection is used in the transformation of potato plants, and transformed plants are isolated.

These transformed plants have reduced SBE I activity, and reduced SBE II activity.

A nucleic acid construct for expression of a starch branching enzyme from the red alga *Gracilaria lemaneiformis* is produced as in Example 3.

This construct (pRBE31) is used for transformation of the transformed potato plants with reduced SBE I and SBE II activity using the xylose selection principle (Haldrup et al. (1998) Plant Mol Biol 37:287-296).

A number of transformed plants are isolated which heterologously express the *Gracilaria lemaneiformis* SBE.

These transformed plants have modified GBE activity.

Starch is prepared from these transformed plants having modified GBE activity, and is found to comprise floridean starch types.

Example 6 - Production of "glycogen-starch types" in potatoes.

The invention provides for the production in certain plants of starch types which are not naturally found in such plants.

In this Example, the invention provides for the production of glycogen starch types in potatoes.

This is accomplished in potato plants by reducing endogenous GBE activity *via* the use of antisense construct expression, and by providing modified GBE activity *via* heterologous expression of bacterial SBE as follows.

Repression of endogenous SBE I and SBE II expression by transformation of potato plants with plasmid pSS28 or plasmid pSS34 as described in Examples 1 and 4 above.

Mannose selection is used in the transformation of potato plants, and transformed plants are isolated.

These transformed plants have reduced GBE activity.

A nucleic acid construct for expression of a starch branching enzyme (a glycogen branching enzyme (GLYBE)) from the bacterium *Escherichia coli* is produced as in Example 3.

This construct (pGLGB7 - Fig. 13) is used for transformation of the transformed plants having reduced GBE activity, using the appropriate selectable marker (kanamycin) as described above.

A number of transformed plants are isolated which heterologously express the *Escherichia coli* GBE glycogen branching enzyme (GLYBE).

These transformed plants have modified GBE activity.

Starch is prepared from these transformed plants having modified GBE activity, and is found to comprise glycogen starch types.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

SEQ I.D. NO. 1

tc agc agt aat ggt gat cgg agg aat gct aat att tct gta ttc ttg
47
Ser Ser Asn Gly Asp Arg Arg Asn Ala Asn Ile Ser Val Phe Leu
1 5 10 15

aaa aaa cac tct ctt tca cgg aag atc ttg gct gaa aag tct tct tac
95
Lys Lys His Ser Leu Ser Arg Lys Ile Leu Ala Glu Lys Ser Ser Tyr
20 25 30

aat tcc gaa tcc cga cct tct aca gtt gca gca tcg ggg aaa gtc ctt
143
Asn Ser Glu Ser Arg Pro Ser Thr Val Ala Ala Ser Gly Lys Val Leu
35 40 45

gtg cct gga acc cag agt gat agc tcc tca tcc tca aca gac caa ttt
191
Val Pro Gly Thr Gln Ser Asp Ser Ser Ser Ser Ser Thr Asp Gln Phe
50 55 60

gag ttc act gag aca tct cca gaa aat tcc cca gca tca act gat gta
239
Glu Phe Thr Glu Thr Ser Pro Glu Asn Ser Pro Ala Ser Thr Asp Val
65 70 75

gat agt tca aca atg gaa cac gct agc cag att aaa act gag aac gat
287
Asp Ser Ser Thr Met Glu His Ala Ser Gln Ile Lys Thr Glu Asn Asp
80 85 90 95

gac gtt gag ccg tca agt gat ctt aca gga agt gtt gaa gag ctg gat
335
Asp Val Glu Pro Ser Ser Asp Leu Thr Gly Ser Val Glu Glu Leu Asp
100 105 110

ttt gct tca tca cta caa cta caa gaa ggt ggt aaa ctg gag gag tct
383
Phe Ala Ser Ser Leu Gln Leu Gln Glu Gly Gly Lys Leu Glu Glu Ser
115 120 125

aaa aca tta aat act ttt gaa gag aca att att gat gaa tct gat agg
431
Lys Thr Leu Asn Thr Phe Glu Glu Thr Ile Ile Asp Glu Ser Asp Arg
130 135 140

atc aga gag agg ggc atc cct cca cct gga ctt ggt cag aag att tat
479
Ile Arg Glu Arg Gly Ile Pro Pro Pro Gly Leu Gly Gln Lys Ile Tyr
145 150 155

gaa ata gac ccc ctt ttg aca aac tat cgt caa cac ctt gat tac agg
527
Glu Ile Asp Pro Leu Leu Thr Asn Tyr Arg Gln His Leu Asp Tyr Arg
160 165 170 175

tat tca cag tac aag aaa ctg agg gag gca att gac aag tat gag ggt
575
Tyr Ser Gln Tyr Lys Lys Leu Arg Glu Ala Ile Asp Lys Tyr Glu Gly
180 185 190

SEQ I.D. NO. 1 (cont)

ggt ttg gaa gct ttt tct cgt ggt tat gaa aaa atg ggt ttc act cgt
623
Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys Met Gly Phe Thr Arg
195 200 205

agt gct aca ggt atc act tac cgt gag tgg gct cct ggt gcc cag tca
671
Ser Ala Thr Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala Gln Ser
210 215 220

gct gcc ctc att gga gat ttc aac aat tgg gac gca aat gct gac att
719
Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asp Ala Asn Ala Asp Ile
225 230 235

atg act cgg aat gaa ttt ggt gtc tgg gag att ttt ctg cca aat aat
767
Met Thr Arg Asn Glu Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn
240 245 250 255

gtg gat ggt tct cct gca att cct cat ggg tcc aga gtg aag ata cgt
815
Val Asp Gly Ser Pro Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg
260 265 270

atg gac act cca tca ggt gtt aag gat tcc att cct gct tgg atc aac
863
Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile Pro Ala Trp Ile Asn
275 280 285

tac tct tta cag ctt cct gat gaa att cca tat aat ggg ata tat tat
911
Tyr Ser Leu Gln Leu Pro Asp Glu Ile Pro Tyr Asn Gly Ile Tyr Tyr
290 295 300

gat cca ccc gaa gag gag agg tat atc ttc caa cac cca cgg cca aag
959
Asp Pro Pro Glu Glu Glu Arg Tyr Ile Phe Gln His Pro Arg Pro Lys
305 310 315

aaa cca aag tcg ctg aga ata tat gaa tct cat att gga atg agt agt
1007
Lys Pro Lys Ser Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser
320 325 330 335

ccg gaa cct aaa att aac tca tac gtg aat ttt aga gat gaa gtt ctt
1055
Pro Glu Pro Lys Ile Asn Ser Tyr Val Asn Phe Arg Asp Glu Val Leu
340 345 350

cct cgc ata aaa aag ctt ggg tac gat gcg gtg caa att atg gct att
1103
Pro Arg Ile Lys Lys Leu Gly Tyr Asp Ala Val Gln Ile Met Ala Ile
355 360 365

caa gag cat tct tat tat gct agt ttt ggt tat cat gtc aca aat ttt
1151
Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe
370 375 380

SEQ I.D. NO. 1 (cont)

```
ttt gca cca agc agc cgt ttt gga act ccc gac gac ctt aag tct ttg
1199
Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Asp Asp Leu Lys Ser Leu
    385                 390                 395

att gat aaa gct cat gag cta gga att gtt gtt ctc atg gac att gtt
1247
Ile Asp Lys Ala His Glu Leu Gly Ile Val Val Leu Met Asp Ile Val
400                 405                 410                 415

cac agc cat gca tca aat aat act tta gat gga ctg aac atg ttt gac
1295
His Ser His Ala Ser Asn Asn Thr Leu Asp Gly Leu Asn Met Phe Asp
                420                 425                 430

ggc aca gat agt tgt tac ttt cac tct gga gct cgt ggt tat cat tgg
1343
Gly Thr Asp Ser Cys Tyr Phe His Ser Gly Ala Arg Gly Tyr His Trp
            435                 440                 445

atg tgg gat tcc cgc ctc ttt aac tat gga aac tgg gag gta ctt agg
1391
Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Asn Trp Glu Val Leu Arg
        450                 455                 460

tat ctt ctc tca aat gcg aga tgg tgg ttg gat gag ttc aaa ttt gat
1439
Tyr Leu Leu Ser Asn Ala Arg Trp Trp Leu Asp Glu Phe Lys Phe Asp
    465                 470                 475

gga ttt aga ttt gat ggt gtg aca tca atg atg tgt act cac cac gga
1487
Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met Cys Thr His His Gly
480                 485                 490                 495

tta tcg gtg gga ttc act ggg aac tac gag gaa tac ttt gga ctc gca
1535
Leu Ser Val Gly Phe Thr Gly Asn Tyr Glu Glu Tyr Phe Gly Leu Ala
                500                 505                 510

act gat gtg gat gct gtt gtg tat ctg atg ctg gtc aac gat ctt att
1583
Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Val Asn Asp Leu Ile
            515                 520                 525

cat ggg ctt ttc cca gat gca att acc att ggt gaa gat gtt agc gga
1631
His Gly Leu Phe Pro Asp Ala Ile Thr Ile Gly Glu Asp Val Ser Gly
        530                 535                 540

atg ccg aca ttt tgt att ccc gtt caa gat ggg ggt gtt ggc ttt gac
1679
Met Pro Thr Phe Cys Ile Pro Val Gln Asp Gly Gly Val Gly Phe Asp
    545                 550                 555

tat cgg ctg cat atg gca att gct gat aaa tgg att gag ttg ctc aag
1727
Tyr Arg Leu His Met Ala Ile Ala Asp Lys Trp Ile Glu Leu Leu Lys
560                 565                 570                 575
```

SEQ I.D. NO. 1 (cont)

aaa cgg gat gag gat tgg aga gtg agt gat att gtt cat aca ctg aca
1775
Lys Arg Asp Glu Asp Trp Arg Val Ser Asp Ile Val His Thr Leu Thr
580 585 590

aat aga aga tgg tcg gaa aag tgt gtt tca tac gct gaa agt cat gat
1823
Asn Arg Arg Trp Ser Glu Lys Cys Val Ser Tyr Ala Glu Ser His Asp
595 600 605

caa gct cta gtc ggt gat aaa act ata gca ttc tgg ctg atg gac aag
1871
Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys
610 615 620

gat atg tat gat ttt atg gct ctg gat aga ccg tca aca tca tta ata
1919
Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser Thr Ser Leu Ile
625 630 635

gat cgt ggg ata gca ttg cac aag atg att agg ctt gta act atg gga
1967
Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly
640 645 650 655

tta gga gga gaa ggg tac cta aat ttc atg gga aat gaa ttc ggc cac
2015
Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His
660 665 670

ccc gag tgg att gat ttc cct agg gct gaa caa cac ctc tct gat ggc
2063
Pro Glu Trp Ile Asp Phe Pro Arg Ala Glu Gln His Leu Ser Asp Gly
675 680 685

tca gta att ccc gga aac caa ttc agt tat gat aaa tgc aga cgg aga
2111
Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp Lys Cys Arg Arg Arg
690 695 700

ttt gac ctg gga gat gca gat tat tta aga tac cgt ggg ttg caa gaa
2159
Phe Asp Leu Gly Asp Ala Asp Tyr Leu Arg Tyr Arg Gly Leu Gln Glu
705 710 715

ttt gac cgg gct atg cag tat ctt gaa gat aaa tat gag ttt atg act
2207
Phe Asp Arg Ala Met Gln Tyr Leu Glu Asp Lys Tyr Glu Phe Met Thr
720 725 730 735

tca gaa cac cag ttc ata tca cga aag gat gaa gga gat agg atg att
2255
Ser Glu His Gln Phe Ile Ser Arg Lys Asp Glu Gly Asp Arg Met Ile
740 745 750

gta ttt gaa aaa gga aac cta gtt ttt gtc ttt aat ttt cac tgg aca
2303
Val Phe Glu Lys Gly Asn Leu Val Phe Val Phe Asn Phe His Trp Thr
755 760 765

SEQ I.D. NO. 1 (cont)

```
aaa agc tat tca gac tat cgc ata ggc tgc ctg aag cct gga aaa tac
2351
Lys Ser Tyr Ser Asp Tyr Arg Ile Gly Cys Leu Lys Pro Gly Lys Tyr
        770                 775                 780

aag gtt gcc ttg gac tcg gat gat cca ctt ttt ggt ggc ttt ggg aga
2399
Lys Val Ala Leu Asp Ser Asp Asp Pro Leu Phe Gly Gly Phe Gly Arg
    785                 790                 795

att gat cat aat gcc gaa tac ttc acc ttt gaa gga tgg tat gat gat
2447
Ile Asp His Asn Ala Glu Tyr Phe Thr Phe Glu Gly Trp Tyr Asp Asp
800                 805                 810                     815

cgt cct cgt tca att atg gtg tat gca cct agt agg aca gca gtg gtc
2495
Arg Pro Arg Ser Ile Met Val Tyr Ala Pro Ser Arg Thr Ala Val Val
                820                 825                 830

tat gca cta gta gac aaa gaa gca gaa gaa gaa gta gca gta gta gaa
2543
Tyr Ala Leu Val Asp Lys Glu Ala Glu Glu Glu Val Ala Val Val Glu
            835                 840                 845

gaa gaa tgaacgaact tgtg
2563
Glu Glu
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Sequence I. D. no. 2

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TTGATGGGGCCTTGAACTCAGCAATTTGACACTCAGTTAGTTACACTCCTATCACTCATC   60
AGATCTCTATTTTTTTCTCTTAATTCAAACCAAGGAATGAATTAAAAGATTAGATTTGAA   120
GGAGAGAAGAAGAAAGATGGTGTATAAACTCTCTGGAGTGCGTTTTCCTACTGTGTCCAT   180
CAGTGTACAATCTAATGGATTCAGCAGTAATGGTGATCGGAGGAATGCTAATGTTTCTGT   240
ATTCTTGAAAAAGCACTCTCTTTCACATCTTGGCGAAAAGTCTTCTTACGATTCCGAATC   300
CCGACCTTCTACAGTTGCAGCATCGGGGAAGGTCCTTGTACCTKGAAHCCMGAGTGATAG   360
CYCYYCATYCCYCAACMGACCAATTTGAGTTCACTGAGACAGCTCCAGAAATTCCCCAGC   420
ATCAACTGATGTAGATAGTTCAACAATGGAACACGCTAGCCAGATTAAAACTGAGAACGA   480
TGACGTTGAGCCGTCAAGTGATCTTACAGGAAGTGTTGAAGAGTTGGATTTTGCTTCATC   540
ACTACAACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAACATTAAATACTTCTGAAGA   600
RACAATTATTGATGAATCTGATAGGATCAGAGAGAGGGGCATCCCTCCACCYTGGACTTG   660
GTCAGAAGATTTATGAAATAGACCCCCTTTTGACAAACTATCGTCAACACCTTGATTACA   720
GGTATTCACAGTACAAGAAAATGAGGGAGGCAATTGACAAGTATGAGGGT   770
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Sequence I. D. no. 3

TTGATGGGGCCTTGAACTCAGCAATTTGACACTCAGTTAGTTACACTGCCATCACTTATC 60

AGATCTCTATTTTTTCTCTTAATTCCAACCAAGGAATGAATAAAAGATAGATTTGTAAAA 120

CCCTAAGGAGAGAAGAAGAAAGATGGTGTATACACTCTCTGGAGTGCGTTTTCCTACTGT 180

TCCATCAGTGTACAAATCTAATGGATTCAGCAGTAATGGTGATCGGAGGAATGCTAATAT 240

TTCTGTATTCTTGAAAAAACACTCTCTTTCACTTGCAGCATCGGGGAAAGTCCTTGTGCC 300

TSGAACCCAGAGTGATAGCTCCTCATCCTCAACAGACCAATTTGAGTTCACTGAGACATC 360

TCCAGAAAATCCCCCAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGCTAGCCA 420

GATTAAAACTGAGAACGATGACGTTGAGCCGTCAAGTGATCTTACAGGAAGTGTTGAAGA 480

GCTGGATTTTGCTTCATCACTACAACTACAAGAAGGTGGTAAACTGGAGGAGTCTAAAAC 540

ATTAAATACTTTTGAAGAGACAATTATTGATGAATCTGATAGGATCAGAGAGAGGGGCAT 600

CCCTCCACCTGGACTTGGTCAGAAGATTTATGAAATAGACCCCCCTTTTGACAAACTATC 660

GTCAACACCTTGATTACAGGTATTCACAGTACAAGAAACTGAGGGAGGCAATTGACAAGT 720

ATGAGGGT 728

Sequence I.D. no. 4

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                          SphI                          PmlI
TTGATGGCAATAATGGAGCATGCGTATTATGCCAGTTTTGGATACCACGTGACTAACTTC   60
AACTACCGTTATTACCTCGTACGCATAATACGGTCAAAACCTATGGTGCACTGATTGAAG
L  M  A  I  M  E  H  A  Y  Y  A  S  F  G  Y  H  V  T  N  F

                                                        ClaI
                                                        BspDI
TTTGCCATCAGCTCTCGCTGTGGGCCACCCGAGGATCTCAAGTACCTCATCGATAAGGCA   120
AAACGGTAGTCGAGAGCGACACCCGGTGGGCTCCTAGAGTTCATGGAGTAGCTATTCCGT
F  A  I  S  S  R  C  G  P  P  E  D  L  K  Y  L  I  D  K  A

                      NcoI                                 NcoI
CATCAGCTCGGATTGTACGTCCYCATGGATGTCGTCCATTCACATGCCTCCAGTAACTCC   180
GTAGTCGAGCCTAACATGCAGGRGTACCTACAGCAGGTAAGTGTACGGAGGTCATTGAGG
H  Q  L  G  L  Y  V  X  M  D  V  V  H  S  H  A  S  S  N  S

                                             ScaI
ATGGACGGTATCAATAACTTCGATGGCACAGACCACCAGTACTTCCATGAAGGAGAACGT   240
TACCTGCCATAGTTATTGAAGCTACCGTGTCTGGTGGTCATGAAGGTACTTCCTCTTGCA
M  D  G  I  N  N  F  D  G  T  D  H  Q  Y  F  H  E  G  E  R

         BbsI                     EarI
GGAAGACATTCGTTGTGGGATTCTAGGCTCTTCAACTATGGTCATTGGGAGGTTCTTCGC   300
CCTTCTGTAAGCAACACCCTAAGATCCGAGAAGTTGATACCAGTAACCCTCCAAGAAGCG
G  R  H  S  L  W  D  S  R  L  F  N  Y  G  H  W  E  V  L  R

TTCCTGCTCTCCAATCTTAGATGGTATATGGAGGAGTATCACTTTGACGGCT           352
AAGGACGAGAGGTTAGAATCTACCATATACCTCCTCATAGTGAAACTGCCGA
F  L  L  S  N  L  R  W  Y  M  E  E  Y  H  F  D  G
```

SEQ I.D. NO. 5

gcccgkgcac ccgkgccgaa aatcaaaccc cgatgatgyc sgcsccgggc gttgaataaa
60

caaaagatac aaacattcac aatccgcatg cttttgtaca taaacttact gtagtgcgcc
120

acgcggatga tgaccatgca ttactcctgc atgatgtgat ggcgccagtt gcgcgggcgc
180

cctgccgtga cgctgcgcag tcgcgagatg tgcacgcgct ggagcacgtg cgcctcgtta
240

aattaaaaat gattcatgtt tactatgcga aaatttaaaa acaaatctga tacaacgatt
300

ccgaagtgtc tcttccgtgg cgtcgctcaa agcgcgaccc caaaatagtg cacaatgcac
360

amagatacat ttttgtacat ctccatatgt acaaagggcc caggcacaag cagcgtccgt
420

tgtgggcgca cgagcccaac agttgccagc cgctcgccaa gcgcctggaa gcggagcacc
480

tccaagcctg cgccttttca atgcacatgc ctattccgtt ggcaacttgt tgcacggccg
540

tgtttctccg aagagcgctg gcgctcatgt gcgcgtggcc atgcgatcga ctctcccgcc
600

gttgcccaaa caaccattct gcccttgtgg ccttaccttc acgctgcgca cttcaccagt
660

tgccattctc tcagcgcgat gatttaaaca acgaatgact tcgctgttca cattgtgatc
720

aaaaaacggt gggtaacaca cgtgtgcctc gtggcgcgtc cagatacgcg gcgtgtgcac
780

agccgcacgc tgcacgcgcg tgccgccacc gctgtcgcgc tcgctgtgtg gctcagttct
840

tcggatcgtg tcagccgtgg cactgcccat tcgccatttc actcgcacac agccgctcat
900

tgcagcccaa ctgcatgtcc tgattcactt aacctccgct ttatttgcgc accccaaatc
960

cacccctcgc acacactcac ttcctccaca cctgcttccc gctcgcctac cgctccccca
1020

tcacttcccc gtcacc atg ggc tcg gaa gac ccc cac tac tcc gcc tgg aag
1072
Met Gly Ser Glu Asp Pro His Tyr Ser Ala Trp Lys
1 5 10

gat aag aaa gac ggc acc gca tgc atc cgc gat gat cgc tac ttg gag
1120
Asp Lys Lys Asp Gly Thr Ala Cys Ile Arg Asp Asp Arg Tyr Leu Glu
15 20 25

SEQ I.D. NO. 5 (cont)

ccc ttt gca gat gcc ctg cgt tat cgc tat gcc aag ttt tcc gag att
1168
Pro Phe Ala Asp Ala Leu Arg Tyr Arg Tyr Ala Lys Phe Ser Glu Ile
30 35 40

ctg tcc gcc atc gag tcc agc gaa aag tcc ctc gac aac ttt tca cgc
1216
Leu Ser Ala Ile Glu Ser Ser Glu Lys Ser Leu Asp Asn Phe Ser Arg
45 50 55 60

gga tat aaa act atg gga atc cag cgc gtc aag gga ggc att cgc tac
1264
Gly Tyr Lys Thr Met Gly Ile Gln Arg Val Lys Gly Gly Ile Arg Tyr
65 70 75

cgc gaa tgg ggc acc cag cgc ccg cga aaa tgt tct tct ttg gcg agt
1312
Arg Glu Trp Gly Thr Gln Arg Pro Arg Lys Cys Ser Ser Leu Ala Ser
80 85 90

tta aca act ggg atc gtc act ctg tcc cta tgc agc gcg atg aat ttg
1360
Leu Thr Thr Gly Ile Val Thr Leu Ser Leu Cys Ser Ala Met Asn Leu
95 100 105

gca tat ggt cct gct tca ttc cgs gat cct gaa ccg ggc gtc ccg gct
1408
Ala Tyr Gly Pro Ala Ser Phe Arg Asp Pro Glu Pro Gly Val Pro Ala
110 115 120

att aag cac gga acc aag gtg aag gct gcc gtt gtg ccc cat cac ggt
1456
Ile Lys His Gly Thr Lys Val Lys Ala Ala Val Val Pro His His Gly
125 130 135 140

ccg tgg ctc gac cgc aac ccg gcg tgg gca acc ttc tgc gtg caa gac
1504
Pro Trp Leu Asp Arg Asn Pro Ala Trp Ala Thr Phe Cys Val Gln Asp
145 150 155

ccg cac aca ttt ctg tat gat acc gtc ttc tgg aac ccc ccg gag aag
1552
Pro His Thr Phe Leu Tyr Asp Thr Val Phe Trp Asn Pro Pro Glu Lys
160 165 170

tac aag tgg att ggt cca gat cac gtc aag cga ccc tct tcg ctt cgt
1600
Tyr Lys Trp Ile Gly Pro Asp His Val Lys Arg Pro Ser Ser Leu Arg
175 180 185

att tac gag tgt cat gtc ggc atg ggc act aac gat cta aag gtc ggc
1648
Ile Tyr Glu Cys His Val Gly Met Gly Thr Asn Asp Leu Lys Val Gly
190 195 200

tca tac aga gag ttt gct gac aac gtt ctt ccg cgt att aag aac act
1696
Ser Tyr Arg Glu Phe Ala Asp Asn Val Leu Pro Arg Ile Lys Asn Thr
205 210 215 220

SEQ I.D. NO. 5 (cont)

ggc tac act gct ttg caa atc atg gcc att atg gag cat gcg tat tat
1744
Gly Tyr Thr Ala Leu Gln Ile Met Ala Ile Met Glu His Ala Tyr Tyr
225 230 235

gcc agt ttt gga tac cac gtg act aac ttc ttt gcc aat mgc tct cgc
1792
Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Asn Xaa Ser Arg
240 245 250

tgt ggg cca ccc gag gat ctc aag tac ctc atc gat aag gca cat cag
1840
Cys Gly Pro Pro Glu Asp Leu Lys Tyr Leu Ile Asp Lys Ala His Gln
255 260 265

ctc gga ttg tac gtc ctc atg gat gtc gtc cat tca cat gcc tcc agt
1888
Leu Gly Leu Tyr Val Leu Met Asp Val Val His Ser His Ala Ser Ser
270 275 280

mmc tcc atg gac ggt atc aat aac ttc gat ggc aca gac cac cag tac
1936
Xaa Ser Met Asp Gly Ile Asn Asn Phe Asp Gly Thr Asp His Gln Tyr
285 290 295 300

ttc cat gaa gga gaa cgt gga aga cat tcg ttg tgg gat tct agg ctc
1984
Phe His Glu Gly Glu Arg Gly Arg His Ser Leu Trp Asp Ser Arg Leu
305 310 315

ttc aac tat ggt cat tgg gag gtt ctt cgc ttc ctg ctc tcc aat ctt
2032
Phe Asn Tyr Gly His Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Leu
320 325 330

aga tgg tat atg gag gag tat cac ttt gac ggc ttc cgt ttc gac ggt
2080
Arg Trp Tyr Met Glu Glu Tyr His Phe Asp Gly Phe Arg Phe Asp Gly
335 340 345

atc act tcc atg ttg tac ctt cat tct ggc att ggt gtg cag ttc act
2128
Ile Thr Ser Met Leu Tyr Leu His Ser Gly Ile Gly Val Gln Phe Thr
350 355 360

ggc aac tat aat gaa tac ttt gga ctg cag gtt gat gtt gat gct tgt
2176
Gly Asn Tyr Asn Glu Tyr Phe Gly Leu Gln Val Asp Val Asp Ala Cys
365 370 375 380

gtt tat atg atg ctt gcc aat aag tta ctt cat gac ttg cat cct gat
2224
Val Tyr Met Met Leu Ala Asn Lys Leu Leu His Asp Leu His Pro Asp
385 390 395

gtg gcc gtg act atc gct gaa gac gtc agc ggc atg cct act ctt tgt
2272
Val Ala Val Thr Ile Ala Glu Asp Val Ser Gly Met Pro Thr Leu Cys
400 405 410

SEQ I.D. NO. 5 (cont)

gtc cct gtt gaa cat ggt gga ctc ggt ttc gac tac cgt ttg gcc atg
2320
Val Pro Val Glu His Gly Gly Leu Gly Phe Asp Tyr Arg Leu Ala Met
415 420 425

gcc att ccc gac atg tgg atc gag gtt ctt gaa aag gag aag gac gag
2368
Ala Ile Pro Asp Met Trp Ile Glu Val Leu Glu Lys Glu Lys Asp Glu
430 435 440

aat tgg aat atg ggt aat att gtg ttc act ctt acc aat cgc cgt tgg
2416
Asn Trp Asn Met Gly Asn Ile Val Phe Thr Leu Thr Asn Arg Arg Trp
445 450 455 460

aac gag aag acc att ggg tac tgc gaa agt cat gat caa gcg ttg gtt
2464
Asn Glu Lys Thr Ile Gly Tyr Cys Glu Ser His Asp Gln Ala Leu Val
465 470 475

ggg gac aag act att gcc ttt tgg ctt atg gat gcc gct atg tac act
2512
Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Ala Ala Met Tyr Thr
480 485 490

gat atg agc tgc gat gga tac cct tcc ccc acc atc gag cgt gga att
2560
Asp Met Ser Cys Asp Gly Tyr Pro Ser Pro Thr Ile Glu Arg Gly Ile
495 500 505

gcg ctg cac aag ttg atc aga ctg ttg acc atg tgc tta tcg ggt gaa
2608
Ala Leu His Lys Leu Ile Arg Leu Leu Thr Met Cys Leu Ser Gly Glu
510 515 520

ggt tac ctt acg ttc atg ggc aat gag ttt ggt cat cca gaa tgg att
2656
Gly Tyr Leu Thr Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile
525 530 535 540

gat ttc ccg cgt gaa gga aac ggc aac tct tac caa cat gca cgt cgt
2704
Asp Phe Pro Arg Glu Gly Asn Gly Asn Ser Tyr Gln His Ala Arg Arg
545 550 555

cgc tgg gat ctt tgc gac aat cag tcg cta cgt tat aag cat ttg tac
2752
Arg Trp Asp Leu Cys Asp Asn Gln Ser Leu Arg Tyr Lys His Leu Tyr
560 565 570

gct ttt gag aag atg gcg cac cct ttg gac gac gaa cat ccg ttc ctc
2800
Ala Phe Glu Lys Met Ala His Pro Leu Asp Asp Glu His Pro Phe Leu
575 580 585

cat ttc cag gca cat cag tat gtc gtt ttg tca cac gaa ggt gac aag
2848
His Phe Gln Ala His Gln Tyr Val Val Leu Ser His Glu Gly Asp Lys
590 595 600

SEQ I.D. NO. 5 (cont)

ctg gtc gtc gtt gag aag ggc gat cgt ctg ctg ttc gtg ttt aac ttc
2896
Leu Val Val Val Glu Lys Gly Asp Arg Leu Leu Phe Val Phe Asn Phe
605 610 615 620

cat cat aat caa tcg tat tct gat tat cgt att ggc act tat tgg ggt
2944
His His Asn Gln Ser Tyr Ser Asp Tyr Arg Ile Gly Thr Tyr Trp Gly
625 630 635

ggt cgg tat aag ttg gtt ctt gat tcg gat ggc acg aac act ggt gga
2992
Gly Arg Tyr Lys Leu Val Leu Asp Ser Asp Gly Thr Asn Thr Gly Gly
640 645 650

cat gga aga gtg cac tgg gat gtg gtc cat acg acg aat act gaa ccg
3040
His Gly Arg Val His Trp Asp Val Val His Thr Thr Asn Thr Glu Pro
655 660 665

tgg cat aac aga cca tac tac ata caa gtg tat gct cct gca cgc acg
3088
Trp His Asn Arg Pro Tyr Tyr Ile Gln Val Tyr Ala Pro Ala Arg Thr
670 675 680

tgc cag gtg tac tat tgc tat gag acg tgg gag gaa gct gcg cag aaa
3136
Cys Gln Val Tyr Tyr Cys Tyr Glu Thr Trp Glu Glu Ala Ala Gln Lys
685 690 695 700

gag ggg aag gca gcc gac gcg caa agc aag cct gca act gta gtg act
3184
Glu Gly Lys Ala Ala Asp Ala Gln Ser Lys Pro Ala Thr Val Val Thr
705 710 715

gag ata gtg gat aag aaa gca gct tct gag aag gct gtc tct ggc gct
3232
Glu Ile Val Asp Lys Lys Ala Ala Ser Glu Lys Ala Val Ser Gly Ala
720 725 730

gag gat gtt gag caa gta gcg tcg aaa cta cgc gca aag ctg acg acg
3280
Glu Asp Val Glu Gln Val Ala Ser Lys Leu Arg Ala Lys Leu Thr Thr
735 740 745

aaa gca gcg gtc aag gct gtt cag aag gaa gct gtg tgagggacat
3326
Lys Ala Ala Val Lys Ala Val Gln Lys Glu Ala Val
750 755 760

tgcttagatg tcggtggggt gttgagctcc catgaggaga acggggtata ctctaacaaa
3386

gtttgtaggc tagatatgtg ggctttctga gagccagtac gaactacaag cttcaagtgg
3446

gtcccgttga tgcaggcggg tttgtttgaa tgagcgttac agtgacaaac cgctgcacag
3506

SEQ I.D. NO. 5 (cont)

atgaatacca tggggtctgc cgacggtagg ttttttgatg ggtatgatat cagttgttct
3566

ttgttttggt ctgttcgtct taggctgaac gctgaaaagc tttctgcgat gcctttgttg
3626

ttgcgatgag tacgaacttg agtattttgt gcagttcgga aacttttgtt gacagatgat
3686

aaacaatact agtcttgtga tttgaatgac catacctca agttggggcg aattcgatat
3746

caagctt
3753

Claims

1. A transformed organism, preferably a transformed plant, having a reduced endogenous starch branching enzyme (SBE) activity, and having a heterologous glucan branching enzyme (GBE) activity.

2. A transformed organism, preferably a transformed plant, having a reduced endogenous starch branching enzyme (SBE) activity, and having a heterologous glucan branching enzyme (GBE) activity, wherein said reduced SBE activity is effected via expression of a nucleotide sequence that is antisense to at least part of a SBE exon.

3. A transformed plant according to claim 2 having a reduced endogenous starch branching enzyme (SBE) activity, and having a heterologous glucan branching enzyme (GBE) activity, wherein said SBE exon is selected from the group consisting of SBE I exon(s), SBE II exon(s), or combinations thereof.

4. A transformed plant having a reduced endogenous starch branching enzyme (SBE) activity, and having a heterologous glucan branching enzyme (GBE) activity, wherein said reduced SBE activity is effected via antisense exon expression, and wherein the antisense exon expression system and the heterologous GBE expression system are present as separate nucleic acid constructs.

5. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises
   i) a starch branching enzyme (SBE), or
   ii) a glycogen branching enzyme (GLYBE), or
   iii) both an SBE and a GLYBE.

6. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a SBE obtainable from an alga.

7. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a SBE obtainable from a red alga.

8. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a SBE obtainable from one or more algae selected from *Gracilaria gracilis* and *Gracilaria lemaneiformis*.

9. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a glycogen branching enzyme (GLYBE).

10. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a GLYBE obtainable from a bacterium.

11. A transformed plant according to any previous claim, wherein said heterologous GBE activity comprises a glycogen branching enzyme (GLYBE), and wherein said GLYBE is obtainable from the bacterium *Escherichia coli*.

12. A method for producing starch with altered characteristics, said method comprising;
    i) providing a plant having reduced endogenous SBE activity, and having heterologous GBE activity,
    ii) propagating the plant of (i), and optionally
    iii) obtaining starch from said plant.

13. Starch obtainable from a transformed plant according to any previous claim.

14. Starch, wherein said starch comprises at least one starch species having a branching pattern not naturally found in a wild-type parent plant.

15. A nucleic acid contruct system capable of directing the expression of all or part of one or more antisense SBE exon(s) and optionally one or more heterologous GBE(s).

16. A nucleic acid contruct system according to claim 14, wherein the antisense SBE and heterologous GBE elements are present as separate nucleic acid constructs.

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Application No:	GB 0006733.0	**Examiner:**	Dr J Houlihan
Claims searched:	1-13	**Date of search:**	30 November 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R):

Int Cl (Ed.7):

Other: WPI, EPODOC, PAJ, AGRICOLA, BIOSIS, CAPLUS, BIOTECHABS, EMBASE, LIFESCI, SCISEARCH, CONFSCI, JICST-EPLUS, CROPB, CROPU

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	WO 99/14314 A	(COMMONWEALTH SCI. & IND. RES. ORG *et.al.*) page 8 lines 25-35; claim 36	1, at least
X	WO 97/22703 A	(E.I. DU PONT DE NEMOURS & CO.) page 7 lines 4-18	1, at least
X	US 6013861	(ZENECA LTD.) column 3 line 65-column 4 line 6; column 4 line 59-column 5 line 8	1, at least
X	Planta Vol. 198 (3) 1996. Flipse E *et.al.* "*Introduction of sense and antisense cDNA for branching enzyme in the amylose-free potato mutant leads to physico-chemical changes in the starch*" pages 340-347, especially page 345 r.h. column lines 21-23		1, at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.